PERICARP STUDIES IN MAIZE. III. THE FREQUENCY OF MUTATION IN VARIEGATED MAIZE PERICARP[1]

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	111
Material and methods	111
Data	112
Comparison of near-self and dark-crown frequencies	116
The theoretical frequency relation between different classes for a constant mutation rate	116
Calculation of comparative mutation rate	117
Comparative rate of mutation	117
SUMMARY	120
LITERATURE CITED	120

INTRODUCTION

Variegated maize pericarp offers good material for studies on mutation as the mutaton rate is very high and significant data can readily be obtained. The studies herein reported were undertaken to determine whether the mutaton rate remained constant through that period in the life history of the plant for which comparative data could be obtained.

MATERIAL AND METHODS

The variegated plants used in these studies were all obtained by outcrossing plants heterozygous for variegated (P^{vv}) and red-cob white (P^{wr}) to white (P^{ww}). Half of the resulting plants were variegated of the factorial constitution $P^{vv}P^{ww}$. The other half were red-cob white and were discarded.

The mutations studied were the mutations from variegated to red. These mutations were tabulated from their somatic expression on the plants themselves. The relative stage in the life history at which the

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mutation took place is indicated by the area affected. Previous work on variegated pericarp (EMERSON 1914, 1917) had shown the visible patches of color on the ear to be due to the same mutational changes which also affected the germ cells. That the changes involved are gene mutations is indicated by the fact that the new forms produced behave as allelomorphs to the parental type and to other members of the same allelomorphic series (ANDERSON 1924).

As described by EMERSON (1917) the mutations observed are of two types:

1. The near-self type in which the color is located in the deeper lying tissue of the pericarp. The glumes of the cob and a patch at the tip of the grain remain white or nearly so. These mutations are heritable and give rise to self-red progeny.

2. The dark-crown type. In this type the color seems to be confined to the epidermis except the tip of the grain which becomes deeply colored. The glumes of the cob, which are made up largely of epidermis, also become red. This type is not inherited presumably because the mutation has taken place in cells which do not give rise to germ cells.

The variegated ears were harvested and examined for mutation areas. Areas covering one grain or more were classified and recorded. Grains with near-self mutation areas covering a considerable fraction of the grain were saved and classified. The remaining grain was shelled and weighed. The number of grains without mutation areas of recorded size was computed from weight and counts of weighed samples.

The plants used in these studies were grown at the Plant Breeding Department of CORNELL UNIVERSITY in 1919 and 1920.

DATA

A tabular presentation of the data on near-self mutation is given in table 1. The original data were tabulated in classes of $\frac{1}{2}$ grain intervals, but the data have been grouped to reduce the number of classes. A similar presentation of the dark-crown data is given in table 2. Dark-crown areas affecting only a fraction of a grain are more difficult to classify so were not recorded.

Many of the larger areas are of indeterminate size because their boundaries do not fall entirely within the areas under observation. They are parts of larger mutation areas including tissue outside of the seed-producing part of the ear. They are the result of mutations taking place earlier than their recorded size would indicate.

TABLE 1

Data on frequency of near-self (heritable) mutations.

PEDIGREE	TOTAL NUMBER OF GRAINS	NUMBER OF GRAINS IN MUTATION AREA														
		¼–¼	¼–½	½–1	1	2	3	4	5	6	7	8	9–16	17–32	33–64	65–128
A 1506	37983	968	188	78	39	20		1		1			1			
A 1507	6009	99	42	16	10	1										
A 1508	11185	228	82	39	17	4	1									
A 1510	32383	834*	229	76	39	15				1						
A 1512	7299	264	25	21	5	3		1								
A 1513	8028	193	20	15	10	3										
A 1514	13993	389	70	28	7	2	1				1					
A 1515	14922	378	124	44	20	5	3									
A 1516	25001	467	112	72	23	2	3	1								
A 1517	25204	1041	140	48	17	13	1									
A 1518	25706	700	111	51	33	10										
A 1519	29790	884	148	86	34	8	3		1							
A 1520	37265	774	173	56	36	8	1	2					2			
A 1740	33159	412	214	105	31	14			1			1	1			
A 1742	1792	15	12	2	1											
A 1746	29768	502	265	98	37	11	1	1		1						
A 1747	15089	214	107	35	17	5	1						1		1	
A 1757	8686	94	32	13	9	1										
A 1762	1067	11	6	1	1	1										
A 1763	4012	55	36	14	6	1										
A 1765	13173	201	70	38	1	7							1			
A 1770	41325	368	133	14	46	7	3						2	1	1	
Ey 843	37580	2008	247	145	68	9	4	1				1	3	1		
Ey 844	782	29	5	0	1											
Ey 847	50102	808	183	64	20	4	3					1				
Ey 848	7631	136	25	11	7	2			1							
Ey 851	48147	1509	386	164	88	23	2	3	3	1		1	3			
Ey 852	3754	115	23	12	6	2	2									
Ey 855	4124	163	25	16	7											
Ey 856	39757	740	195	59	45	6	1				1		1			
Ey 860	901	32	8	3	2											
Ey 862	2052	86	19	7	4	1										
Ey 864	2123	77	19	10	5	1							1			
Ey 868	1191	41	18	2	9	1										
Ey 871	1062	34	7	1	1	1										
Ey 874	33233	1281	299	122	72	20	4	1	1		1			1		
Ey 875	1580	38	11	4	1											
Ey 876	543	26	2	1	0											
Ey 877	2296	67	10	7	8	4		1								
Ey 878	29541	1428	181	67	50	13	6	2								
Ey 883	654	19	3	2	0											
Ey 886	4513	160	32	10	10	5	2									1
Ey 890	1277	33	8	4	3											
Total	695682	17921	4045	1661	846	233	42	14	7	4	3	4	16	3	2	1

* Estimated. Observed number not recorded.

TABLE 2

Data on frequency of dark-crown (non-heritable) mutations.

PEDIGREE	TOTAL NUMBER OF GRAINS	NUMBER OF GRAINS IN MUTATION AREA																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17-32	33-64	65-128	129-256	257-512
A 1506	37983	748	42	12	10	4	4			2	1				1		1	3	2			
A 1507	6009	130	2		1		2															
A 1508	11185	278	20	7	3			1												1		
A 1510	32383	808	41	14	6	6		2		1					2			1				
A 1512	7299	93	10	1							1											
A 1513	8028	68	6	1																		
A 1514	13993	139	12	1	1	1	1	1														
A 1515	14922	187	4	2		1	1	1										2				1
A 1516	25001	377	11	4	3	1	1	1	3	1					1			1			1	1
A 1517	25204	447	23	7		1	1			1			1	1		1			1			
A 1518	25706	525	29	4	1	2	1	1		1	1			1	1	1				1		2
A 1519	29790	498	31	6	2					2				1	1				1	1		
A 1520	37265	588	25	10	4	4	3	1			1		1	1				3				
A 1740	33159	484	32	11	4	3	1		1	1					1		1	1				
A 1742	1792	6	1		1			1			1							2				
A 1746	29768	318	13	4	3	2	3			1								1				
A 1747	15089	188	14	3	1				1													2
A 1757	8686	45	4	1	1														2			1
A 1762	1067	8	1																			
A 1763	4012	38	1				1															
A 1765	13173	152	6	6		1	1															
A 1770	41325	526	31	6	3	2	1		1	1		1						2				

TABLE 2 (continued)

Data on frequency of dark-crown (non-heritable) mutations.

PEDIGREE	TOTAL NUMBER OF GRAINS	NUMBER OE GRAINS IN MUTATION AREA																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17-32	33-64	65-128	129-256	257-512
Ey 843	37580	291	35	41	7	2	6	1	1	1			1	1	1		1	3	1			
Ey 844	782	2																				
Ey 847	50102	214	13	3	2	1	2								2	1						
Ey 848	7631	35	3	2	1								1									
Ey 851	48147	783	65	26	17	6	6	2		1	3	3				1		1	2		2	
Ey 852	3754	59	6	4		1	1															
Ey 855	4124	94	6		3	1										1						
Ey 856	39757	334	51	9	5	4	2	1			1	1	1	1				2	1			
Ey 860	901	4	1																			
Ey 862	2052	38	7	3																		
Ey 864	2123	31	4										1									
Ey 868	1191	7	1			1																
Ey 871	1062	13	1																			
Ey 874	33233	658	36	15	6	2	3	2	2	1	1	2			1			4	2			
Ey 875	1580	26																				
Ey 876	543	6																				
Ey 877	2296	28	4	3			1															
Ey 878	29541	313	35	10	7	3	1	3		1	1	1	3			1		1		1		
Ey 883	654	3	4	1																		
Ey 886	4513	54	7	2		2	1	1		2												
Ey 890	1277	21	2										1									
Total	695682	9665	640	192	92	51	44	19	9	17	11	8	10	6	11	6	3	27	12	4	3	7

COMPARISON OF NEAR-SELF AND DARK-CROWN FREQUENCIES

Table 3 gives a comparison of the frequency of occurrence of equivalent sized near-self and dark-crown mutation areas. The frequencies from tables 1 and 2 are grouped into classes such that the limits of one class are twice those of the preceding. This is done to avoid the extremely low frequencies of the individual classes of large area.

TABLE 3

Comparison of frequencies of near-self (heritable) and dark-crown (non-heritable) mutations.

MUTATION AREA	NEAR-SELF	DARK-CROWN
0.5– 1	2509	9665
1 – 2	233	640
3 – 4	56	284
5 – 8	18	123
9 – 16	16	72
17 – 32	3	27
33 – 64	2	12
64 –128	1	4
129 –256	0	3
257 –512	0	7
Total	2838	10837

The frequencies for the dark-crown series average about 3.8 times as great as those for the corresponding near-self classes. This may mean that the mutation rate is actually this much greater in those meristem cells which give rise to the tissues affected by the dark-crown mutations. But since the method of growth of the tissues concerned may not be comparable, this cannot be considered proved.

THE THEORETICAL FREQUENCY RELATION BETWEEN DIFFERENT CLASSES FOR A CONSTANT MUTATION RATE

If the mutation rate were constant the number of mutation areas of small size would be more frequent than those of large because there would have been more cells present which might have mutated. Also the numerical class interval chosen is not one that gives an equivalent length of time for mutations to take place. The exact numerical relation cannot be calculated due to the irregularity of growth. But a calculation based on regular multiplication of cells will approximate the true relationship sufficiently closely for comparison with the observed data. A mutation

affecting twice the area of another may be assumed to have occurred one cell generation earlier when the number of cells was only one-half as great. The time interval, expressed in cell generations, may be calculated on the same basis. An error, probably very small, is introduced in the near-self series by growth in the third dimension during the period under observation.

On the above basis, the relative number of available cells at the time of mutation varies inversely as the size of the mutation area, and the time interval varies as the differences between the logarithms of the numerical sizes of the mutation areas.

CALCULATION OF COMPARATIVE MUTATION RATE

The ratios of the observed frequencies to the calculated relative frequencies give us a measure of the relative mutation rate for the period of several cell generations during which the recorded mutations took place. The calculation of this comparative mutation rate may be simplified by a process which is equivalent to making the theoretical equal to unity and multiplying the observed frequencies by the appropriate factor. The simplified calculation is made as follows: (a) The observed frequencies are multiplied by their respective class values. (b) These products are then summed into classes having equal logarithmic intervals. The interval chosen is such that the numerical limits of one class are twice those of the preceding. This interval is such that each class then represents a cell generation. This summation greatly condenses the long series of low frequency classes.

The number of units of area available for mutation is slightly altered by the fact that once an area has mutated it is no longer available for mutation. This error can be corrected by dividing each product by the difference between the total number of grains and the number included in the larger mutation areas. The change due to this correction is not sufficient to alter the calculated rates more than a few percent but for the sake of completeness both the uncorrected and corrected figures are given in the tables and graphs which follow.

COMPARATIVE RATE OF MUTATION

The comparative rate of mutation calculated for the near-self series is given in table 4. The rate of mutation is expressed as the number of cells mutating per hundred thousand during a period equivalent to one cell generation. A graph of the mutation rate for the near-self series is given

TABLE 4

Mutation rate of near-self (heritable) mutations expressed as the number of mutations per hundred thousand cells during one cell generation.

MUTATION AREA	RATE	CORRECTED RATE
0.12– 0.25	644.0	668.7
0.25– 0.5	290.7	300.0
0.5 – 1.0	360.4	365.4
1.0 – 2.0	67.0	67.7
2.0 – 4.0	26.2	26.4
4.0 – 8.0	16.1	16.2
8.0 –16.0	26.6	26.8
16.0 –32.0	9.5	9.5
32.0 –64.0	11.4	11.4

in figure 1. The graph is arranged so that successive cell generations read from left to right. The last class is of little value because of difficulty of classifying. The irregularity in the curve near the right end is largely due to the fact that the class limits adopted do not conform to the original

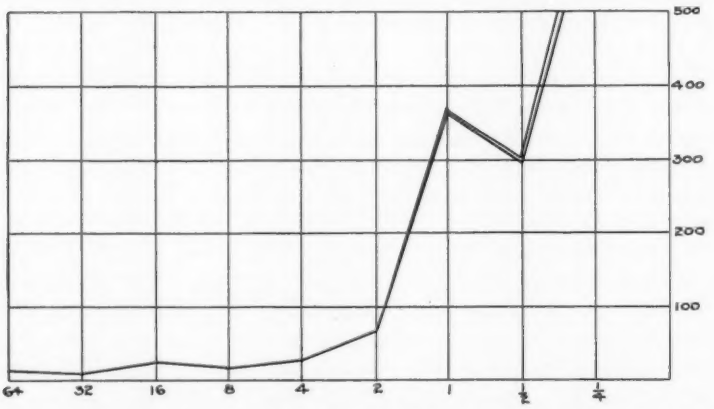

FIGURE 1.—Graph of near-self mutation rate from table 4. Size of mutation areas plotted as abscissae, mutation rates as ordinates.

classification. Table 5 and figure 2 give the corresponding data for the dark-crown series. The curve is smoother because there is no discrepancy in the summation of classes. Mutation areas affecting more than 64 grains for the near-self and more than 128 grains for the dark-crown series are omitted as most of these extended beyond the limits of the ear on which they occurred and were consequently of indeterminate size.

TABLE 5

Mutation rate of dark-crown (non-heritable) mutations expressed as the number of mutations per hundred thousand cells during one cell generation.

MUTATION AREA	RATE	CORRECTED RATE
1	1389.3	1408.7
1 – 2	184.0	190.4
2 – 4	135.7	137.1
4 – 8	104.1	105.0
8 – 16	120.9	121.8
16 – 32	93.1	93.7
32 – 64	82.7	83.1
64–128	58.5	58.8

The rate of mutation for both series shows a great increase within the period covered by these observations. The increase is probably a gradual one, as the sharp changes in the graphs are at least largely due to difficulties encountered in the classification of mutation areas of small size. The

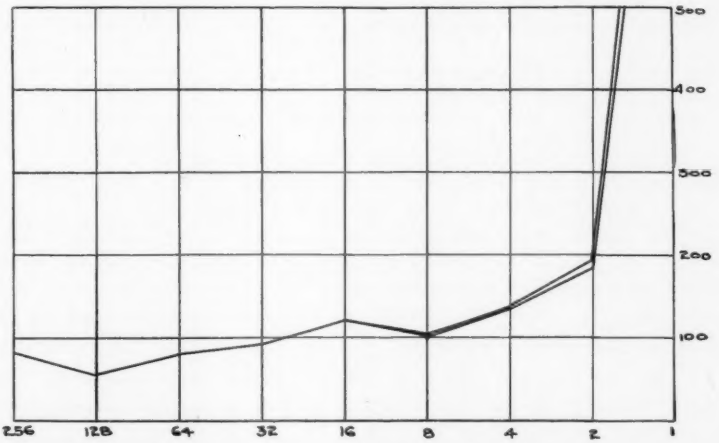

FIGURE 2.—Graph of dark-crown mutation rate from table 5. Size of mutation areas plotted as abscissae, mutation rates as ordinates.

values recorded for the larger areas are a little too large due to the inclusion of mutation areas which were not completely included in the seed-bearing part of the ear on which they occurred.

As the recurrent mutations involved in variegation are perhaps not comparable to other gene mutations, it cannot be inferred from these data

that other gene mutations will show a similar increasing frequency during ontogeny. In this connection, it is well to remember that in the conspicuous example of recurrent change in the bar gene of *Drosophila melanogaster*, STURTEVANT has shown that the changes are due to unequal crossing over in the immediate vicinity of the bar gene (STURTEVANT 1925). This is in marked contrast to the mutations recorded in this paper which occurred in somatic tissue long before the maturation divisions.

SUMMARY

Somatic mutations to red in variegated maize pericarp were classified as to the area affected. The near-self (heritable) and dark-crown (non-heritable) series of mutations were recorded separately.

The dark-crown mutations were about 3.8 times as frequent as the near-self mutations affecting equal areas.

Both the near-self and dark-crown series show an increasing rate of mutation during the period of ontogeny covered by these studies.

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ALCOHOL AND BODY WEIGHT IN THE ALBINO RAT

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This paper deals with one phase, effect upon body weight, of an experiment designed to test the effects of alcohol, both germinal and somatic, upon the albino rat. The data given cover ten successive generations of alcoholic administration. An introductory paper by HANSON and HANDY (1924) gives in detail the methods employed.

Suffice it here to say that: the fume tank method devised by STOCKARD was used in administering the alcohol; treatment was begun in each generation when the animals were twenty days of age and continued until they were one hundred days old, except in the first generation where the treatment continued for a period of one year; the treatment might be described as severe, lasting each day until the animals were unable to stand upon their feet; the general environmental conditions for test and control rats were identical—the alcoholic treatment being the single differential between the two groups; both treated and control animals were the descendants of a single pair of WISTAR INSTITUTE semi-inbred rats (Tyler strain); all matings throughout the experiment were sister-by-brother within the litter; the animals were weighed to a tenth of a gram at birth, at twenty days of age and every ten days thereafter until the last weighing at age one hundred days; all weighings were made by the senior author thereby reducing the personal equation to a minimum.

After the first generation which was small in numbers an attempt was made to base each body weight constant upon at least fifty rats. In practice, however, this was not always possible, the number falling below in some cases and rising considerably above in others. It is believed that in the rigid character of the controls, the number of treated generations, the strictly inbred character of the blood-lines, and the total number of animals (1825 at twenty days of age) involved, this experiment may contribute something to the alcohol problem.

Table 1 gives the data for the totals of the ten generations of control and test animals. The means are based on large numbers of rats, and these numbers are given to the left of each mean in the table. The data for the males and females are given separately.

Males: In males at twenty days of age the control mean is only 0.27 of a gram greater than the corresponding mean in the treated. As this

difference is less than its probable error it seems clear that alcohol fumes, administered to rats over a period of five years and including ten generations, has not impaired the ability of these animals to produce young of equal weight with those of the controls. It will be recalled that treatment

TABLE 1

A table showing body weight constants for the total ten generations at various ages.

AGE IN DAYS	NUMBER RATS	CONTROL	NUMBER RATS	TREATED	DIFF. / P. E
		Males			
20	408	25.17±0.22	490	24.90±0.21	——
40	392	64.39±0.58	434	58.57±0.59	7.09
60	364	113.71±1.06	374	102.76±1.01	7.52
80	317	156.70±1.40	317	145.55±1.44	4.82
100	248	190.85±1.70	227	177.31±1.86	5.42
		Females			
20	454	24.14±0.22	473	25.07±0.21	3.10
40	412	59.66±0.52	421	55.89±0.50	5.24
60	402	99.85±0.86	386	91.01±0.85	7.31
80	374	130.11±1.00	324	126.30±1.08	2.59
100	266	150.79±1.25	170	144.18±1.68	3.19

began at twenty days of age, so that comparisons between treated and controls at this age are based upon rats which have not themselves been treated. Any inherited growth deficiency due to alcoholization of parents and grandparents should be evident at this age. HANSON and HEYS also have shown elsewhere that in litter size and birth weight the offspring of the treated are fully equal to those of the controls.

After the weighing at twenty days those rats in the alcoholic line of descent received their first treatment in the fume tanks and both treatment and weighings were continued until one hundred days of age was attained. The comparison between the two means at every subsequent weighing shows a large significant difference, reaching its maximum at sixty days where the difference is 7.52 times its probable error. This difference at every age above twenty days is in favor of the controls. The conclusion that alcoholic treatment has adversely affected the growth rate seems valid—in other words, descendants with an alcoholic ancestry weigh just as much at birth and at twenty days as the corresponding controls, but these same descendants show a marked falling off in body weight soon after they themselves become the subjects of treatment.

However, as above indicated, these same alcoholic-stunted animals for ten successive generations do not lose their inherent capacity to produce young which at the age of twenty days have normal body weight. Therefore, the effects of alcohol on body weight as shown by these data do not go beyond the soma.

Females: At the ages of forty to one hundred days inclusive the results with females are identical with those of the males and the discussion above applies equally here.

At twenty days the situation is different in this respect: that the young of treated ancestry actually have a larger mean weight by 0.93 of a gram than the controls. At all later ages, due to the direct action of the alcohol, the means of the treated are significantly lower than those of the controls.

Not only does an alcoholic parentage not inhibit growth up to twenty days of age, but actually in the case of females, due to selective elimination of inferior germ cells, differential prenatal mortality or some other unknown cause, produces twenty-day offspring with a significantly greater mean body weight.

It is worthy of notice that at twenty days in both males and females the mean body weight of treated and controls shows a difference of less than a gram although the two groups have been carried in separate lines of descent for a period of over five years.

MACDOWELL treated white rats with alcohol primarily with a view to testing their learning reactions in a Watson maze. Data on growth and fertility were taken at the same time and these have been published separately. The paper on growth (1922) discusses the effect of alcohol on body weight in several groupings of his animals variously described as "Treated rats," "Untreated rats from treated parents," "Untreated rats from untreated parents and treated grandparents," and "Treated rats from treated parents."

His first and last groups are most comparable to our experiment and they alone will be discussed. In the group termed by MACDOWELL, "Treated rats," his conclusion is that the treatment of white rats with maximum doses of alcohol tends to retard growth as compared with their untreated brothers and sisters. His data are in every particular, as regards this one group, identical with ours. In his group known as "Treated rats from treated parents," he found no significant differences between treated and controls. These latter data are based on thirty-one treated rats and thirty-four controls. Our own experiment might be described as treated rats from treated parents and grandparents for ten generations and in-

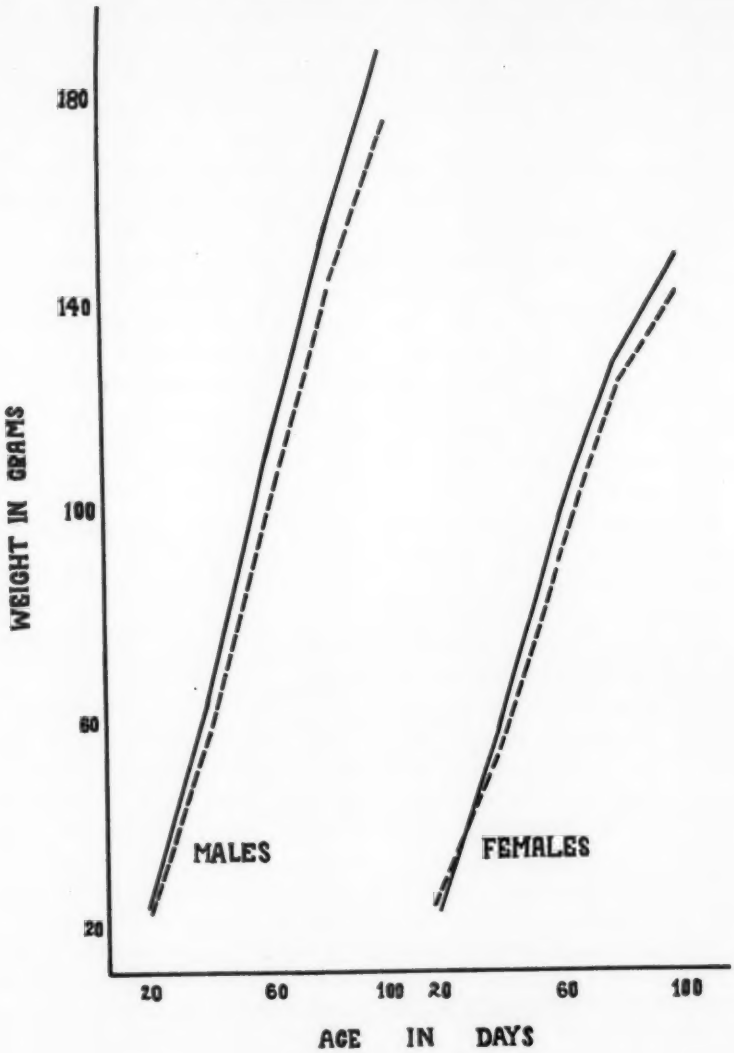
180
140
100
60
20
WEIGHT IN GRAMS
MALES
FEMALES
20
60
100
20
60
100
AGE IN DAYS

FIGURE.—1

volves nearly two thousand rats. That our results in this particular instance are not in agreement with MACDOWELL'S may well be due to the difference in the magnitude of the two experiments.

ARLITT (1919) claimed that alcohol retarded body growth and that "the defective body weight acquired by alcoholized rats is also inherited." MACDOWELL has ably criticised ARLITT'S methods and apparently undermined her conclusions. Our data, based on the same species, are, of course, further refutation if any be needed.

STOCKARD and PAPANICOLOAU (1917) found that in guinea pigs the normal offspring weigh more and for a time grow more rapidly than the young of alcoholic pigs.

PEARL (1917) describes the effects of both ethyl and methyl alcohol on chickens. After fifteen months of treatment the alcoholic birds were 9.9 percent heavier than untreated control birds of the same average age, and this increase in weight of the treated birds is apparently due entirely to deposition of body fat and not a fatty infiltration of any of the visceral organs.

CONCLUSION

1. Alcohol has a retarding effect upon the growth rate of albino rats.
2. This effect is not in any degree transmitted to their offspring even after ten successive generations of exposure to the fumes.

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PARTHENOGENESIS AND THE INHERITANCE OF COLOR PATTERNS IN THE GROUSE LOCUST, *TELMATETTIX AZTECUS* SAUSSURE[1]

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MATERIAL AND METHODS

Specimens of grouse locusts, identified by Doctor A. N. CAUDELL as *Telmatettix aztecus* Saussure, were secured along Shoal Creek, Austin, Texas, in August, 1922. By breeding analyses it was determined that there were four elementary patterns as follows: (1) A mottled gray closely resembling the +/+ of *Apotettix eurycephalus* (NABOURS 1925), and the ensemble of indistinguishable factors responsible for it, are represented by the symbol +/+; (2) a dense black extending over the anterior pronotum and on the femora of the jumping legs is indicated by the letters *Bl;* (3) a grayish white over the anterior pronotum and lateral lobes, resembling *C*, of *Paratettix texanus* (NABOURS 1917), although the posterior pronotum and legs are quite yellow, is represented by the letter *C*; (4) a dark greenish gray over the pronotum, including vertex, eyes and femora, with lighter lateral lobes, ranging almost to white, is designated by the letter *H*. In October, 1923, new specimens were secured from the same region, along Shoal Creek, and these included a new pattern; (5) a dull brick red covering the vertex, eyes, pronotum and posterior femora. This is designated by the letter *R*.

In January, 1923, Mr. DAVIS sent some specimens of this species from Pasadena, California. Only two individuals, females +/+ and +/*C*, respectively, lived to be mated. They both produced offspring from males of the Texas stock. None of the progeny from the +/+, Pasadena female, was bred further. Three individuals from the +/*C* female (Pasadena) were bred and their progenies were widely used in later matings. It is to be noted, however, that none of the females producing parthenogenetic offspring, described farther on, had an ancestry which traced back to either of these Pasadena females.

All adult individuals of this species (*Telmatettix aztecus*) have had long wings and pronota, as against *P. texanus* and *A. eurycephalus*, individuals of which frequently have had short wings and pronota (NABOURS 1914, 1917, 1925).

[1] Contribution No. 96 from the Department of Zoology, Agricultural Experiment Station, KANSAS STATE AGRICULTURAL COLLEGE.

They were bred casually and in small numbers during the year 1922-23, rather intensely and in considerable numbers in 1923–24, and only in small numbers since (till 1927).

They are bred in the greenhouse, and the mating jars used are the regular 8″×12″ glass cylinders set in pots of earth and covered with screen wire lids. The food, consisting mainly of the filamentous algae, is placed on small earthern pots partly buried upside down in the soil (NABOURS 1914, 1925).

The length of time required for breeding a generation varies with the time of the year and conditions of environment, the best season being the spring and early summer months. On the average, four succeeding generations a year may be bred in the greenhouse at Manhattan, Kansas. On hatching, the young are transferred, about 25 each, to larger similar cages. When of sufficient size for the color patterns to be easily distinguished, preferably during the third instar, they are recorded. As they become adult, the desired matings are made, and those remaining are again recorded, and preserved in 95 percent alcohol, in glass tubes sealed with a torch.

The experimental breeding of this species is much more difficult than is that of *P. texanus* and still more arduous than of *A. eurycephalus*. A large proportion of the matings are not productive, due usually to the early death of one or both individuals. During the whole period 1922–27, only 116 of the 187 matings made were productive. During the one year of most intensive breeding, May, 1923, to June, 1924, only 53.9 percent of the matings were productive; the range throughout the months of that year is indicated in table 1.

TABLE 1

Matings made compared with number productive—year 1923–24.

MONTH	MATINGS MADE	PRODUCTIVE MATINGS
June	4	2
July	2	1
August	5	3
September	18	7
October	28	13
November	5	1
December	2	1
January	1	1
February	9	3
March	8	3
April	21	17
May	12	10
Totals	115	62
53.9 percent productive		

The number of individuals recorded fell far short of the number of young hatched and transferred from the mating jars. During the whole period of five years, only 50.6 percent or 2850 of the 5634 offspring transferred, were finally recorded. However, during the year June, 1923, to July, 1924, when better attention was given them, 55.19 percent of those transferred had their color patterns recorded. Table 2 shows the distribution of the mortality throughout that year.

TABLE 2

Number transferred compared with number recorded—year 1923–24.

MONTH	NUMBERS TRANSFERRED	NUMBERS RECORDED
July	214	110
August	64	30
September	0	0
October	154	61
November	317	96
December	28	10
January	50	19
February	313	173
March	155	94
April	264	169
May	504	272
June	1,448	904
Totals	3,511	1,938
55.19 percent recorded		

SEGREGATION OF FACTORS IN THE MATED INDIVIDUALS

(Males on left, females on right of hyphen)

The segregation of the factors of all the individuals heterozygous for the color patterns is shown in table 3. Referring to the table; beginning at the left with the item *Bl*/*C*, for example, all males bred gave 166 carrying *Bl*:165 carrying *C*; the females gave 192 *Bl*:174 *C*; both males and females gave 358 *Bl*:339 *C*, in a total of 697 gametes.

In this paper, as in a previous one (NABOURS 1925), the symbol + (or +/+) is made to serve, as it were, a double role. In tables 3 and 4, and wherever so used, + opposite a symbol for a dominant factor (for example +/*C*), refers simply to the normal recessive allelomorph of that factor. In case none of the dominant factors is present, +/+ refers to the ensemble of indistinguishable factors that produces the mottled gray pattern, common to all species of the Tettigidae so far used in the experiments, and which has been found generally recessive to the more striking patterns. This mottled gray pattern, +/+, is much more

abundant among all the species observed in nature than are the more prominent dominant patterns. Therefore, it might be very well called the "wild type," though all the dominant patterns are also found in nature.

TABLE 3

A summary of the segregation of factors of heterozygous males and females of Telmatettix aztecus.

HETEROZYGOTES	SEGREGATION OF FACTORS IN MALES		SEGREGATION OF FACTORS IN FEMALES		SEGREGATION OF FACTORS IN MALES AND FEMALES COMBINED		TOTAL GAMETES
+/*C*	+	*C*	+	*C*	+	*C*	
	153	157	213	228	366	385	751
+/*Bl*	+	*Bl*	+	*Bl*	+	*Bl*	
	312	276	96	96	408	372	780
+/*H*	+	*H*	+	*H*	+	*H*	
	67	53	67	60	134	113	247
+/*R*	+	*R*	+	*R*	+	*R*	
	131	121	145	167	276	288	564
Bl/*C*	*Bl*	*C*	*Bl*	*C*	*Bl*	*C*	
	166	165	192	174	358	339	697
Bl/*H*	*Bl*	*H*	*Bl*	*H*	*Bl*	*H*	
	0	1	32	24	32	25	57
Bl/*R*	*Bl*	*R*	*Bl*	*R*	*Bl*	*R*	
	0	0	99	124	99	124	223
C/*H*	*C*	*H*	*C*	*H*	*C*	*H*	
	211	215	96	68	307	283	590
C/*R*	*C*	*R*	*C*	*R*	*C*	*R*	
	149	130	58	62	207	182	389
H/*R*	*H*	*R*	*H*	*R*	*H*	*R*	
	33	39	24	26	57	65	122
Totals	1222	1147	1022	1029	2244	2176	4420

In addition to the data summarized in the table above, there was one mating of a male +/*C* to a female +/*C*. This gave 44+/*C* and *C*/*C*:14 +/+ individuals, a close approximation of the 3:1 ratio.

PARTHENOGENESIS

Parthenogenesis has been found to occur in *Apotettix eurycephalus* (NABOURS 1919, 1925) and *Paratettix texanus* (NABOURS and FOSTER, abstract, 1925).

Virgin females of *T. aztecus* were kept by themselves from time to time to test them for parthenogenesis (NABOURS and SNYDER 1924). Two or three females of a kind were placed together in each cage, except once when one female was alone.

Of 15 groups of females thus separated from males, 12 gave from 1 to 112 offspring. The first four items of table 4 are hardly worthy of a place in the table, because early death prevented complete records, but they were undoubtedly cases of parthenogenesis. In another cage, in which two females had been kept, unhatched eggs were found. The other eight items gave quite definite results. The two $+/C$ females, (9), table 4, that gave 49 females, $+/+$, and 53 females, C/C, were among the highest producers parthenogenetically (112 offspring transferred) that have been recorded among the individuals of any of the three species.

The one C/C female produced parthenogenetically, item (6), in table 4, was mated to an H/R male, and gave C/H 5–7:C/R 3–7. Another C/C female from a parthenogenetic progeny, item (9), table 4, was mated to a $+/Bl$ male, and gave $+/C$ 0–4:Bl/C 2–2.

TABLE 4

Parthenogenesis in Telmatettix aztecus
(Males on the left; females on the right; figure on right of second hyphen indicates sex not recorded).

HETEROZYGOUS FEMALES	PARTHENOGENETIC OFFSPRING
(1) 2 *Bl/C*	*Bl/Bl* 0–0–1
(2) 2 *Bl/C*	*Bl/Bl* and *C/C* 0–3–12
(3) 2 *+/Bl*	*+/+* 0–0–1
(4) 2 *+/C*	*C/C* 0–0–2
(5) 2 *Bl/C*	*Bl/Bl* 0–1
(6) 2 *+/C*	*C/C* 0–1
(7) 2 *Bl/C*	*C/C* 0–1
(8) 1 *+/Bl*	*+/+* 0–8, *Bl/Bl* 0–8
(9) 2 *+/C*	*+/+* 0–49, *C/C* 0–53
(10) 3 *C/H*	*C/C* 0–7, *H/H* 0–5
(11) 3 *+/R*	*+/+* 0–1, *R/R* 0–1
(12) 3 *+/C*	*+/+* 0–3, *C/C* 0–3

DISCUSSION OF PARTHENOGENESIS

PEACOCK and HARRISON (1926) have analyzed the data of the experiments showing parthenogenesis in *Apotettix eurycephalus* (NABOURS 1919, 1925) and found from the records that all the unmated individuals that gave offspring could be traced back to hybrids from specimens of a Mexican variety crossed with individuals of a variety from Texas. They

concluded, apparently with good reasoning, that this evidence supported their previously stated hypothesis (PEACOCK and HARRISON 1925) that parthenogenesis was consequent upon hybridity. But in *Telmatettix aztecus*, herein reported, *the individuals that gave parthenogenetic progeny were all descended directly and exclusively from specimens captured on a very small area along Shoal Creek, Austin, Texas.* None of the progeny from the Pasadena, California, specimens was mixed with those that gave offspring parthenogenetically.

However, the fact that parthenogenesis was, in these cases, confined to stocks that had come from one small area does not eliminate the possibility of hybridization, even there. This is a rather widely distributed species, and the chances for individuals of varieties to be carried by the wind, or other agencies, from place to place should not be overlooked.

An extraordinary range in markedly differing elementary color patterns, and a vast array of possibilities for combinations into pattern complexes (see plates, NABOURS 1917, 1923, 1925) are exhibited by practically all the species of the Tettigidae. They undoubtedly also have other equally, or more potent characteristics, even though less readily discernible, in just as great, or greater variety and hybrid complexity. Therefore, the results of the parthenogenetic breeding of *T. aztecus*, reported here, can hardly be made to bear on the hypothesis advanced by PEACOCK and HARRISON, certainly not in the direction of refuting it. In fact, it does not appear now just how this interesting hypothesis might be put to a critically experimental test with any of the species of the grouse locusts, from whatever small area breeding stock might be collected, for they are all probably complexly hybridized.

CYTOLOGY

In *Paratettix texanus*, *Apotettix eurycephalus*, *Acrydium granulatum*, *Tettigidea parvipennis* and other grouse locusts (Tettigidae), there have been found uniformly thirteen spermatogonial chromosomes (HARMAN 1915, ROBERTSON 1916). Cytological preparations for the study of spermatogenesis in *T. aztecus*, prepared with the aid of Doctor MARY T. HARMAN, exhibit six and seven chromosomes in the nuclei of the secondary spermatocyte divisions.

SUMMARY

1. A series of four factors for dominant color patterns and their normal recessive have been analyzed. It appears that these factors are confined to the one series, or one pair of chromosomes, and that they are alterna-

tive, or so closely linked that no crossing over has occurred during the experiments.

2. Gynogenetic parthenogenesis occurs, in which segregation of the factors of heterozygotes takes place as in bisexual reproduction.

3. Females descended directly from individuals collected on a very small area gave offspring parthenogenetically. The results of this experiment, for the reasons given, are, therefore, neutral with respect to the hypothesis of PEACOCK and HARRISON that parthenogenesis is consequent upon hybridity.

4. The chromosome numbers have been found to be six and seven in the secondary spermatocyte division cells.

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LINKAGE STUDIES IN RICE[1] *

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	133
Experiments and results	135
Relation between the awn and the glutinous character	135
Linkage between the T_y t_y pair and the G_l g_l pair of factors	137
Relation between the pericarp color and the glutinous character	139
Inheritance of glume length and its relation with the g_l, p_{r_1}, and p_{r_2} factors	140
Inheritance of spikelet length and its relation with other characters	141
Inheritance of apiculus color and its relation to other characters	146
Relation between stigma color and other characters	149
Relation between leaf-sheath color and other characters	151
Linkage between stigma color and leaf-sheath color	153
Relation between ligule color and other characters	156
Relation of hull color to other characters	157
DISCUSSION OF RESULTS	159
SUMMARY	163
ACKNOWLEDGMENT	164
LITERATURE CITED	164
APPENDIX	165

INTRODUCTION

According to the chromosome theory, all genes on the same chromosome are linked, unless crossing over takes place. If this postulate is true, we should expect to find no more linkage groups than there are haploid chromosomes in the variety or species in question. Thus far, *Drosophila melanogaster* is the only species in the animal kingdom in which all known genes have been found to fall into one or another of the four linkage groups, each probably corresponding to one of the four haploid chromosomes. In the plant kingdom, *Zea mays* is the only species in which the linkage relations of Mendelian characters have been studied extensively.

In rice, the common species, *Oryza sativa L.*, has twenty-four chromosomes (KUWADA 1910, NAKATOMI 1923). We should expect, therefore, to find twelve linkage groups in the common races of rice. Investigations in this respect, however, have just begun. PARNELL (1917) observed the

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association between purple lining of the internode and purple glumes, and also between purple stigma and purple axil, whereas green internode and glumes are associated with purple stigma and axil. He further observed (1922) that purple pericarp color belongs to the same pattern. No satisfactory data, however, have been published regarding the chromosomal relations of the genes responsible for the characters just mentioned. NAGAI (1921) observed close association between the purple awn and the reddish-brown testa. HECTOR (1922) showed that the color of the pericarp is due either to the same factor which is responsible for ligule color or to a factor completely linked with it, and that the fact that a few plants were found with colored ligules and white grains is evidence in favor of the latter view. HECTOR (1922) further found that certain color characters on the vegetative parts of the plant are grouped in patterns or systems which are inherited together, segregating as if they were due to a single factor or due to the same interacting factors. In four cases, however, he observed that some patterns were altered. (1) In the cross Noachur × Pookhi, the pattern "colored internode and stigma" was altered three times out of 1,199 plants examined, giving three plants in F_2 with "colored stigma and green internode." (2) In the same cross, the pattern "colored leaf-sheath and apiculus" changed twice out of 1,199 plants giving two plants in F_2 with "colored apiculus and green leaf-sheath." (3) In the cross Bailabkri × Pookhi the pattern "colored leaf-sheath, internode and stigma" was altered twenty-four times out of 4,687 plants examined, giving twenty-four plants with "colored leaf-sheath and stigma but green internode." (4) In the cross Agartollah × C_{25}, the pattern "colored leaf-sheath, pulvinus, auricles, internode, glumes, apiculus" changed once out of 4,669 plants examined, namely, one plant was found with color in the glumes and apiculus, but with green leaf-sheath, internode, pulvinus and auricles. In all the cases thus far reviewed, while there is some indication of association between certain characters, no linkage group is definitely established.

In three instances, however, the linkage relation seems to be clear. The first of these is between the factor for awn color and the glutinous gene. In the cross, Tamanishiki × Shinriki, TAKAHASHI (1923) found that the dominant factor for awn color (*R*) is coupled with the non-glutinous factor (*U*), giving about twenty-one percent crossing over. The exact percentage of crossing over, however, is not certain, for NAGAI (1926) found 21.7 percent crossing over in one cross and 14.3 percent crossing over in another cross. Of course, the latter case may be concerned with a different factor. The second instance of linkage is given by YAMAGUCHI

(1926) who found that the factor for apiculus color (*S*) is coupled with the non-glutinous gene giving about 20–22 percent crossing over. Just recently, YAMAGUCHI (1927) found that the factor (*F*) for flowering time is also linked with the glutinous gene. The exact locus, however, is not certain.

The present studies were started in the winter of 1924. Some twenty-five factors were studied and their chromosomal relations determined so far as practicable. Since it is not practical to make backcrosses with this plant, the linkage relations were analyzed exclusively from data obtained from the F_2 generation. The varieties used have been briefly described in an earlier paper by the author (CHAO), but a few important characters are pointed out in the following list.

Variety	*Characters*
100	Glutinous endosperm; awnless; colorless apiculus and glumes.
200	Non-glutinous endosperm; long awn; tawny apiculus and glumes.
300	Glutinous; colorless apiculus, stigma and leaf-sheath.
400	Non-glutinous; colored apiculus, stigma and leaf-sheath.
600	Non-glutinous; colorless apiculus, stigma and leaf-sheath; red brown pericarp; colorless ligule, auricle, and internode; light hull with brown furrows.
800b	Glutinous; colored apiculus, stigma and leaf-sheath; purple pericarp; purple ligule, auricle and internode; light hull without brown furrows.
4269	Glutinous; long spikelet and long glumes; colorless apiculus, stigma and leaf-sheath; colorless ligule, auricle and pulvinus; purple pericarp.
4957	Non-glutinous; short spikelet and short glumes; red apiculus; purple stigma; leaf-sheath purple lined; colorless ligule, auricle and pulvinus; white pericarp.

EXPERIMENTS AND RESULTS

Relation between the awn and the glutinous character

Concerning the inheritance of the awned and awnless character in rice, YAMAGUCHI (1926) reported a simple Mendelian 3:1 ratio. NAGAI (1926) reported three cases, one segregating in a ratio of three awned: one awnless; another segregating in a ratio of one awned: three awnless: and the third, a ratio of fifteen awned: one awnless.

The writer found a case similar to the one last mentioned. The F_1 was fully awned like the awned parent. The F_2 population consisted of four types. One type was fully awned and another fully awnless like the original parents. Of the two new types, one had awns on most of the spikelets, while the other had awns on a few spikelets only, as shown in figure 1. These four types were designated as *fully awned*, *mostly awned*, *rarely awned*, and *fully awnless*, respectively. They occurred in a ratio of

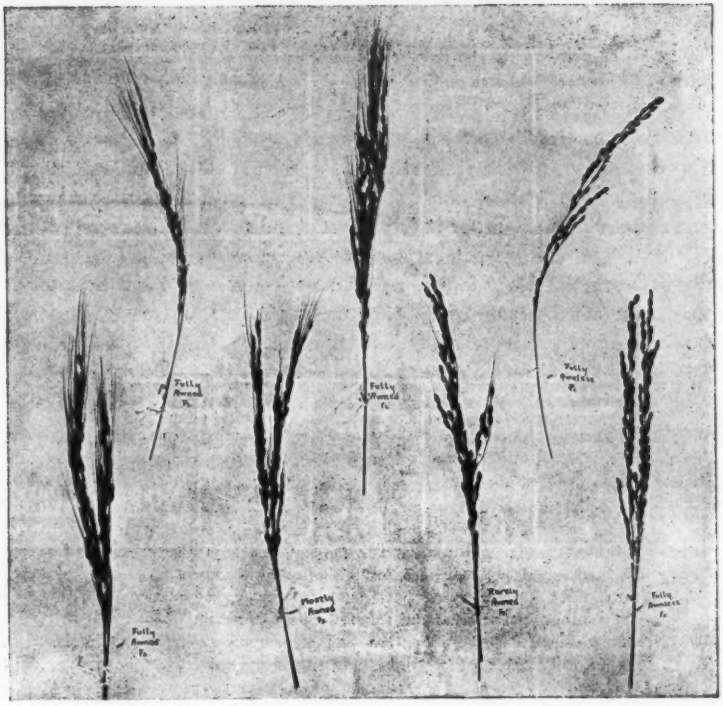

FIGURE 1.—Showing P_1, F_1, and F_2 generations of 101×205, with particular reference to the segregation of the awn character.

12:1:2:1. When all the awned types were classified together, however, the ratio of awned to awnless approaches very closely to 15:1, as shown in table 1.

TABLE 1

Segregation of fifteen awned: one awnless in the F_2 generation of (101×205) D.

PHENOTYPES	OBSERVED	*Calculated 12:1:2:1*	OBSERVED	*Calculated 15:1*	DEVIATION
Fully awned	319	*326.16*			
Mostly awned	29	*27.18*	406	*407.70*	−1.7
Rarely awned	58	*54.36*			
Fully awnless	29	*27.18*	29	*27.18*	1.82

$$\frac{\text{Dev.}}{\text{P. E.}} = \frac{1.82}{3.41} = 0.53$$

These facts are explained on the basis of two pairs of factors $A_{n_1}a_{n_1}$ and $A_{n_2}a_{n_2}$, both being concerned with the production of the awn character. A_{n_2} is considered to be weaker in its action than A_{n_1}. So, A_{n_1} can produce the fully awned type with or without A_{n_2}. A_{n_2} in double dose may produce the mostly awned class, but in single dose, it will only produce the rarely awned type. The fully awnless type is due to the double recessive constitution. The interpretation that one of these two factors is weaker in its action than the other is supported by the fact that among the mostly awned and rarely awned types of F_2 plants the frequency of awned spikelets on the early or late panicles of the same plant may vary according to environmental conditions, while among the fully awned or fully awnless types the phenotypic expression is not so easily subject to environmental influence.

These two pairs of factors $A_{n_1}a_{n_1}$ and $A_{n_2}a_{n_2}$ are independent of the glutinous pair, $G_1\ g_1$, as shown in table 2. It is to be noted that among the F_2 population of 434 plants, 349 were non-glutinous and 85 glutinous, showing a deficiency of glutinous grains that is about 3.86 times the probable error.

TABLE 2

Independent segregation between factors $A_{n1}a_{n1}$, $A_{n2}a_{n2}$ and G_1g_1.

PHENOTYPES	OBSERVED	*Calculated 45:3:15:1*	$\frac{(O-C)^2}{C}$
Non-glutinous awned	327	*305.10*	1.57
Glutinous awned	78	*101.70*	5.52
Non-glutinous awnless	22	*20.34*	0.13
Glutinous awnless	7	*6.78*	0.01
	434	*434*	$7.23=X^2$

P=0.0718

The deviation from expectation on the basis of independent segregation is not large, if we take into consideration the significant deficiency of the glutinous plants involved.

Linkage between the $T_y\ t_y$ pair and the $G_1\ g_1$ pair

In this particular material, when the panicle first emerges from the leaf-sheath, the apiculus, the glumes, and the awn, if present, are green like the other parts of the spikelet. As the spikelet is filled up by the developing grain, the three parts concerned gradually develop color through successive shades from Pale Orange Yellow, Light Orange Yellow up to

Tawny (Ridgway) or even brighter and more glassy than Tawny at maturity. This is designated as the "tawny character," and the absence of it is non-tawny or colorless.

In inheritance, the tawny character is completely dominant over non-tawny in the F_1 generation. In the F_2, the writer obtained a simple 3:1 ratio, as shown in table 3.

TABLE 3

F_2 segregation of tawny non-tawny from the cross (101×205)D.

	TAWNY	NON-TAWNY	TOTAL
Observed	339	99	438
Calculated 3:1	*328.5*	*109.5*	*438*
Deviation		−10.5	

$$\frac{\text{Dev.}}{\text{P.E.}} = \frac{10.5}{6.11} = 1.71$$

Since the tawny color appeared on three parts of the spikelet and no crossing over was noticed in a population of 438 F_2 plants, it is very probable that the tawny character is due to one allelomorphic pair of factors rather than to several different genes completely linked. This allelomorphic pair is designated as T_y t_y.

Breeding data presented in table 4 show that the T_y factor is coupled with the G_1 factor. On the basis of independent segregation, we should

TABLE 4

Coupling between G_1 and T_y
(Gametic ratio $r:s:s:r = 4.48:1:1:4.48$)

	NON-GLUTINOUS TAWNY	NON-GLUTINOUS COLORLESS	GLUTINOUS TAWNY	GLUTINOUS COLORLESS	TOTAL
Observed	309	43	30	58	440
Calculated	*293.11*	*36.39*	*36.39*	*73.44*	*440*
Deviation	15.89	6.61	−6.39	−15.44	

$X^2 = 5.05$
$P = 0.17$

expect 247.5 non-glutinous tawny plants, 82.5 non-glutinous colorless plants, 82.5 glutinous tawny plants, and 27.5 glutinous colorless plants.

But the observed data deviated very widely from the expectation. By EMERSON'S (1916) method, the gametic ratio is found to be:

$$r:s:s:r = 4.48:1:1:4.48$$

From this, the zygotic ratio is calculated. The percentage of crossing over is about 16.59 percent. Considering the significant deficiency of the glutinous plants involved, the calculated ratios are fairly close to the observed ones.

Relation between the pericarp color and the glutinous character

The pericarp color of rice varies from pure white, grey-brown, red to purple. Several investigators notably THOMPSTONE (1915), PARNELL and AYYANGAR (1917), IKENO (1918), NAGAI (1921), and HECTOR (1922), have reported that red and white colors form a simple Mendelian pair segregating in a 3:1 ratio in F_2, red being dominant over white. PARNELL *et al* (1917), however, found a case where red and white segregated in a 9:7 ratio. PARNELL (1922) reported that purple and white also form a Mendelian pair segregating in a 3:1 ratio and that purple×red gave a ratio of 12 purple: 3 red: 1 white in F_2. KATO and ISHIKAWA (1921) found a case where red×white gave a ratio of 9 red:3 yellow:4 white. All these facts seem to indicate that there are two factors concerned with the production of pericarp color.

I found that the Chinese Imperial rice has a red pericarp color which also behaved as a simple Mendelian dominant when crossed with a white variety. This allelomorphic pair is designated as P_{r_1} p_{r_1}. Further data show that P_{r_1} is independent of the factor G_1, as shown in table 5.

TABLE 5

Independent segregation between G_1 and P_{r1} (600×100).

	NON-GLUTINOUS RED PERICARP	NON-GLUTINOUS WHITE PERICARP	GLUTINOUS RED PERICARP	GLUTINOUS WHITE PERICARP	TOTAL
Observed	96	31	37	15	179
Calculated					
9:3:3:1	*100.62*	*33.54*	*33.54*	*11.18*	*179*
Deviation	−4.62	−2.54	3.46	3.82	

In another cross between 4269 and 4957, the former being purple and the latter white, a ratio of 15 colored:1 colorless was obtained, showing that there are two factors concerned. One of these two genes in this case

is presumably the same as P_{r_1} and the other is designated as P_{r_2}. Both of them are independent of the glutinous gene, as shown in table 6.

TABLE 6

Independent segregation between P_{r1} and P_{r2} and g_1 from the cross 4269×4957.

	NON-GLUTINOUS COLORED	NON-GLUTINOUS COLORLESS	GLUTINOUS COLORED	GLUTINOUS COLORLESS	TOTAL
Observed	515	36	147	11	709
Calculated 45:15:3:1	*498.15*	*33.21*	*166.05*	*11.07*	*709*
Deviation	16.85	2.79	−9.05	−0.07	

Inheritance of glume length and its relation with the g_1, p_{r_1}, and p_{r_2} factors

At the base of the spikelet there are two small lance-shaped structures called glumes. In common varieties, the glumes are very short, about one-third as long as the lemma and palea. But there are some varieties in which the glumes are as long as the lemma and palea. In inheritance, PARNELL *et al* (1917) and NAGAI (1921) have reported that the short glume is dominant to the long glume giving a simple 3:1 ratio in F_2.

The writer found a case where the short glume × long glume gave a 15:1 ratio, long glume being recessive, as shown in table 7.

TABLE 7

Segregation for glume length in the cross 4269 (long)×4957 (short).

	SHORT GLUME	LONG GLUME	TOTAL
Observed	674	43	717
Calculated 15:1	*672.15*	*44.81*	*717*
Deviation		−1.81	

$$\frac{\text{Dev.}}{\text{P.E.}} = \frac{1.81}{4.37} = 0.41$$

Since the observed ratio is remarkably close to 15:1, undoubtedly there are two duplicate factors concerned with the production of the glume length. These two pairs of duplicate genes are designated as G_1g_1 and G_2g_2.

Further data clearly indicate that g_1 and g_2 are independent of the glutinous gene g_l, as shown in table 29. The observed data approach the calculated ratios very closely, considering the deficiency of the glutinous plants involved. Furthermore, the duplicate genes G_1 and G_2 have no chromosomal relations with factors P_{r_1} and P_{r_2}, as shown in table 30.

Inheritance of spikelet length and its relation with other characters

The spikelet length varies with different varieties. The variety, 4957, has spikelets varying from 3.5–4.9 mm in length with an average of 4.13 mm. The variety 4269 has spikelets varying from 7.3–10.3 mm in

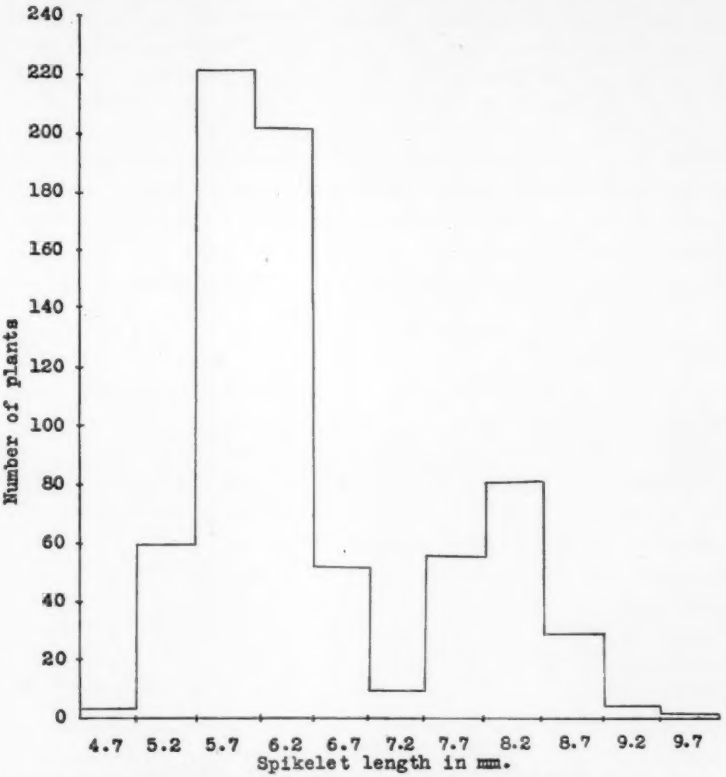

FIGURE 2.—Showing segregation for spikelet length in F_2 of cross 4269×4957.

length with an average of 8.81 mm. The variation within each of these varieties follows a normal frequency curve.

In the cross, 4269×4957, made by the writer at the CROWLEY RICE EXPERIMENT STATION, 1925, ten F_1 plants were obtained, each showing a similar intermediate type of spikelet ranging from 3.9–6.4 mm long with an average of 5.33 mm in length. In the F_2 generation, segregation for the

spikelet length took place, but the spikelets on the same plant were uniform, as expected. Six spikelets taken from the different parts of a panicle in a random fashion were measured and their average length was taken to represent the spikelet length of the plant in question. That this method of sampling actually gives a representative value is proved by the fact that when all the 95 spikelets of a panicle from the plant (Hg_{l_1}) were measured, their average was 5.66 mm approaching very closely to the respective average lengths of several samples of six spikelets each taken at random from that same panicle, namely 5.86, 5.3, 5.75, 5.64, and 5.66. In a population of 718 F_2 plants, the spikelet length ranged from 4.7 to 9.7 mm, as shown in table 8. When the different class values were plotted against the frequency of each class, we obtained a distinctly bimodal curve (figure 2). Since the dividing point of the two groups on the curve

TABLE 8

Showing segregation for spikelet length in F_2 from the cross 4269×4957.

GENERATION	CLASS CENTERS (IN MM) AND FREQUENCIES													NUMBER OF PLANTS	NUMBER OF SPIKELETS
	3.7	4.2	4.7	5.2	5.7	6.2	6.7	7.2	7.7	8.2	8.7	9.2	9.7		
P_1 (4957)	1	3												4	282
P_1 (4269)											4			4	362
F_1				10										10	851
F_2			2	60	222	202	52	9	56	81	29	4	1	718	6 for each plant

is clearly at the class center, 7.2, which is exactly the length of the shortest spikelet of the long parent, and since the longest spikelet of either the short parent or of the F_1 is never over 6.5 mm in length, it appears legitimate to place the nine plants of the class (7.2) in the long group. In so doing, the F_2 population is divided into two phenotypes, one with short spikelets and the other with long spikelets in almost exactly a 3:1 ratio, as shown in table 9.

TABLE 9

Segregation for short and long spikelet in F_2 from 4269×4957.

	SHORT SPIKELET	LONG SPIKELET	TOTAL
Observed	538	180	718
Calculated 3:1	*538.5*	*179.5*	*718*
Deviation		0.5	

The data clearly indicates that the spikelet length, in this material at least, is due to one allelomorphic pair of factors which may be designated as S_s s_s.

The S_p s_p pair of genes are not linked with the G_1 g_1 pair as shown in table 31, nor with P_{r_1} p_{r_1} and P_{r_2} p_{r_2} as shown in table 32.

We have seen, in the preceding pages, that glume length depends upon two independent duplicate factors G_1g_1 and G_2g_2, the short glume being dominant and that the spikelet length depends on one allelomorphic pair of genes, S_p s_p. When the variety, 4957, having short spikelet and short glumes, was crossed with the variety 4269, having long spikelet and long glumes, all F_1 plants had short glumes and intermediate spikelets. In the F_2 generation, two new types occurred in addition to the two grandparental types, as illustrated in figure 3.

The F_2 population consists of 538 plants having short spikelet and short glumes, one plant having short spikelet and long glumes, 134 plants having long spikelet and short glumes, and 45 plants having long spikelet and long glumes, as shown in table 10. On the basis of three independent factors, we should expect the corresponding classes of F_2 plants to be 504.45, 33.63, 168.15, and 11.21, respectively. But this is not the case; the observed data show a great excess of the two parental types. If we assume that one of the glume factors is the same as the spikelet factor, the calculated ratios would be 12:0:3:1, giving the class frequencies 538.50, having short glumes and short spikelet, none having short spikelet with long glumes, 134.625, having long spikelet with short glumes, and 44.875 having long spikelet and long glumes. The expectation on this assumption fits the observed data very well, except that the single plant with short spikelet and long glumes is not accounted for, as shown in table 10.

TABLE 10

Relation between the glume length (15:1) and the spikelet length (3:1).

PROGENIES	SHORT SPIKELET SHORT GLUME	SHORT SPIKELET LONG GLUME	LONG SPIKELET SHORT GLUME	LONG SPIKELET LONG GLUME	TOTAL
4269×4957A	37	0	3	6	
" B	48	0	11	4	
" C	52	1 (CSTG)	13	3	
" D	58	0	14	5	
" E	45	0	6	5	
" F	76	0	22	8	
" G	68	0	16	5	
" H	4	0	4	0	
" I	73	0	18	6	
" J	77	0	27	3	
Observed	538	1	134	45	718
Independent 45:3:15:1	504.45	33.63	168.15	11.21	717.44
Independent 12:0:3:1	538.50	0	134.625	44.875	718

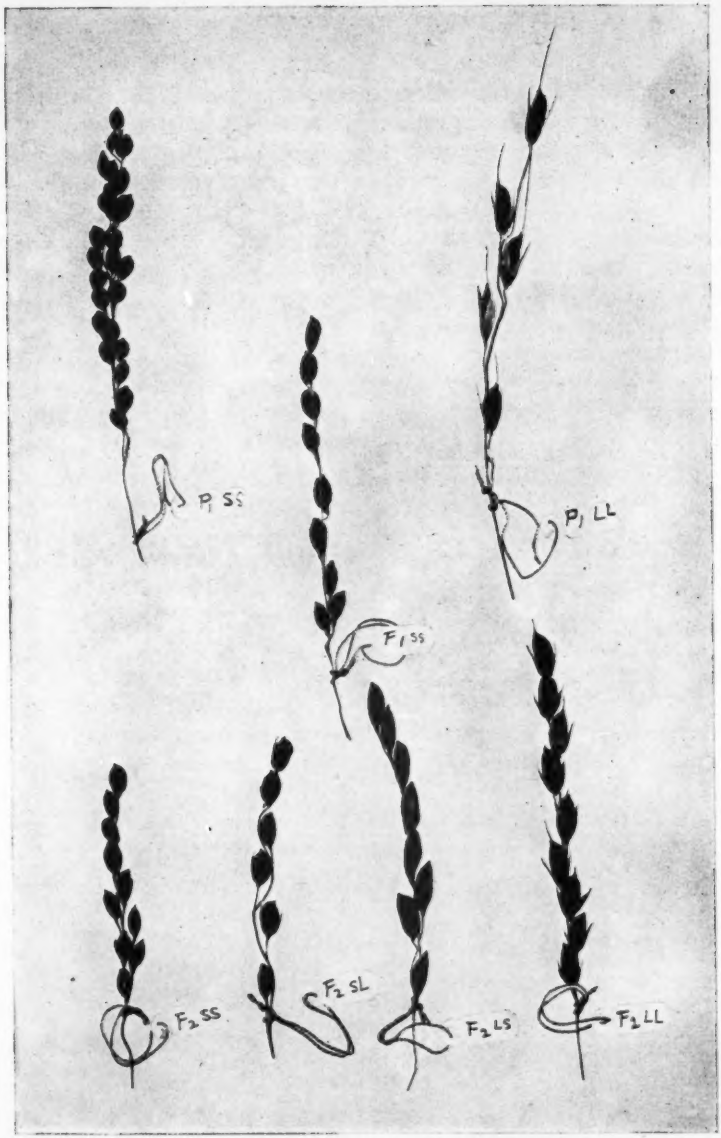

FIGURE 3.—P_1 *ss* = The paternal parent having short spikelet and short glumes. P_1 *LL* = The maternal parent, having long spikelet and long glumes. F_1 *ss* = Hybrid, having short or intermediate spikelet and short glumes. F_2 *ss* = One of 538 F_2 plants, having short spikelet and short glumes. F_2 *sL* = The only F_2 plant which had short spikelets and long glumes. This combination is a result of crossing over. F_2 *Ls* = One of the 134 F_2 plants having long spikelets and short glumes. F_2 *LL* = One of the 45 plants having long spikelets and long glumes.

The same difficulty occurs if we assume complete linkage between one of the glume factors and the spikelet factor.

So far as the evidence goes, the best explanation, in the writer's opinion, may be obtained on the basis of close linkage between one of the duplicate factors for glume length and the factor for spikelet length. Here, it may be arbitrarily assumed that g_2 is closely linked with s_p. On this assumption, we can calculate the gametic ratio.

If only the factors G_1g_1 and $S_p\ s_p$ had been concerned in this cross, the F_2 distribution would be represented by the general formula,

$$\frac{a}{3r^2+2(s^2+2rs)}:\frac{b}{s^2:2rs}:\frac{c}{s^2+2rs}:\frac{d}{r^2} \qquad (1)$$

Where $r:s:s:r$ is any gametic series and a, b, c, and d are the phenotypes, G_1S_p, G_1s_p, g_1S_p, and g_1s_p, respectively.

But when the G_2g_2 pair of genes is also involved, as is the case in this cross, r = the non-crossover gametes $G_1G_2S_p$, $g_1G_2S_p$, $G_1g_2s_p$, and $g_1g_2s_p$; and s = crossover gametes $G_1G_2s_p$, $g_1G_2s_p$, $G_1g_2S_p$, and $g_1g_2S_p$. Combinations of these gametes in all possible ways grouped according to phenotypes may be represented by the general formula,

$$\frac{a}{12r^2+11(s^2+2rs)}:\frac{b}{3r^2+4(s^2+2rs)}:\frac{c}{s^2+2rs}:\frac{d}{r^2} \qquad (2)$$

From formula (2), we get the following four equations:

$a = 12r^2+11(s^2+2rs)$ = Short spikelet and short glumes
$b = 3r^2+4(s^2+2rs)$ = Long spikelet and short glumes
$c = 2rs+s^2$ = Short spikelet and long glumes
$d = r^2$ = Long spikelet and long glumes

From these four equations, we can determine the gametic ratio directly from the observed zygotic series. Thus, we get $a+b+c+d = 16r^2+16c$

$$16r^2 = a+b+d-15c$$

$$r^2 = \frac{a+b+d-15c}{16}$$

$$r = 0.25\sqrt{a+b+d-15c} \qquad (3)$$

Also,

$$16(s^2+2rs) = a+b+c-15r^2$$

$$s^2+2rs = \frac{a+b+c-15r^2}{16}$$

Adding r^2 (or d) to both sides, we get,

$$r^2+2rs+s^2=\frac{a+b+c+d}{16}$$

$$r+s=0.25\sqrt{a+b+c+d}$$

$$s=0.25\sqrt{a+b+c+d}-r \qquad (4)$$

By substituting the observed data of the four phenotypes for a, b, c, and d, in the formulae (3) and (4), we get,

$$r=6.6225$$
$$s=0.0750$$

If we take $s=1$, the ratio of $\frac{r}{s}=\frac{88.3}{1}$

By substituting the values of r and s in the four equations, we obtain the expected frequencies of the four phenotypes, which fit the observed data unusually closely, as shown in table 11.

TABLE 11

Showing coupling between g_2 and s_p (crossover = 1.11 percent).

	SHORT SPIKELET SHORT GLUMES	LONG SPIKELET SHORT GLUMES	SHORT SPIKELET LONG GLUMES	LONG SPIKELET LONG GLUMES	TOTAL
Observed	538	134	1	45	718
Calculated (linkage)	*537.5*	*135.63*	*0.99*	*43.87*	
Deviation	0.5	−1.63	0.01	1.13	

$X^2=0.0533$. When $X^2=1$, $P=.801253$

Since this is a coupling phase, the crossover between g_2 and s_p will be,

$$\frac{s}{r-s}\times 100=1.11 \text{ percent.}$$

Inheritance of the apiculus color and its relation with other characters

The apex of the lemma and palea is colored in many varieties. This localized color spot at the upper tip of the spikelet is here spoken of as the apiculus color. The inheritance of this character has been studied by several investigators. HECTOR obtained a ratio of three colored apiculus to one colorless in 1913 and another ratio of 27:37 in 1916. Besides, HECTOR (1922) reported two new conditions, one of which segregated in a 9:7 ratio and the other in a 15:1 ratio.

In crosses between different varieties, the writer obtained various ratios in the F_2 generations, namely, 3:1, 9:7, 15:1, 27:37, and 162:94, indicating that there are at least four genetic factors responsible for the production of the apiculus color. The data are presented in table 12.

TABLE 12

Showing segregation of colored and colorless apiculus in different ratios in F_2 populations of different crosses.

	CROSSES AND THE PARENTAL TYPES	F_1	F_2 SEGREGATION		CLOSEST RATIO	EXPECTED F_2		DEVIATION	SYMBOLS
			Colored	Colorless		Colored	Colorless		
1	Colorless×colored 300×400	Colored	77	65	9:7	79.83	62.09	2.91	CC A_{p_1}, A_{p_2}
2	Colorless×colorless 300×600	Colored	356	499	27:37	360.45	493.95	5.05	Cc A_{p_1}, A_{p_2}
3	Colorless×colored 600×400	Colored	330	190	162:94	328.86	190.82	−0.82	A_{p_1}, Cc A_{p_2}, A_{p_3}
4	Colored×colorless 800×600	Colored	255	22	15:1	259.65	17.31	4.69	A_{p_2}, A_{p_3}
5	Colorless×colored 4269×4957	Colored	567	176	3:1	557.25	183.75	7.75	A_{p_1}

That variety 300, though colorless, actually carries some factor is shown by the fact that when it was crossed with another colorless variety 600 (see cross No. 2), the F_1 had colored apiculus. In the F_2, a ratio of 27:37 was observed, showing at least three factors were involved. This situation may be explained, if the two parental types have the following genetic constitutions:

$300 = g_1\, g_1\, CC\, a_{p_1}\, a_{p_1}\, a_{p_2}\, a_{p_2}\, a_{p_3}\, a_{p_3}$ (glutinous colorless)

$600 = G_1\, G_1\, cc\, A_{p_1}\, A_{p_1}\, A_{p_2}\, A_{p_2}\, A_{p_3}\, A_{p_4}$ (non-glutinous colorless

Where A_{p_1}, A_{p_3} and C are complimentary factors for apiculus color.

In cross No. 1 (300×400) the 9:7 ratio clearly indicates that two factors are concerned in the production of the apiculus color, these being designated as A_{p_1} and A_{p_2}. The situation can be explained by assuming the genetic constitutions of the parents as follows:

Parent $300 = g_1\, g_1\, CC\, a_{p_1}\, a_{p_1}\, a_{p_2}\, a_{p_2}$

Parent $400 = G_1\, G_1\, CC\, A_{p_1}\, A_{p_1}\, A_{p_2}\, A_{p_2}$

Where C = chromogen, and A_{p_1} and A_{p_2} are complementary for the apiculus color.

That the variety 600 actually carries at least one apiculus factor differing from those in the variety 400 is proved by cross No. 3, which gave a ratio of 162:94, showing that four factors are involved. The situation may be explained on the bases of the foregoing genetic constitutions assumed for varieties 400 and 600, respectively.

Cross No. 4 gave a ratio of 15:1, showing that there are two factors involved, each of them alone producing apiculus color. These factors may be designated as A_{p_5} and A_{p_6}. As no further crosses were made, the assumption must be considered as a tentative one.

Cross No. 5 gave a simple 3:1 ratio, showing that only one factor pair was involved. This factor pair is different from A_{p_1}, A_{p_2}, and A_{p_3}, in that none of the latter alone can produce the apiculus color. Furthermore, this new factor is linked with the glutinous gene as will be shown later. This gene is designated as A_{p_4}. It may be that one of the two duplicate factors involved in cross No. 4 is the same as A_{p_4}.

Factors A_{p_1} and A_{p_2} are independent of the glutinous gene as shown in table 13.

TABLE 13

Showing independent segregation between factors A_{p_1}, A_{p_2} and g_1 (307×410).

	COLORED APEX NON-GLUTINOUS	COLORLESS APEX NON-GLUTINOUS	COLORED APEX GLUTINOUS	COLORLESS APEX GLUTINOUS	TOTAL
Observed	65	51	12	14	142
Calculated 27:21:9:7	*59.67*	*46.41*	*19.89*	*15.47*	*142*
Deviation	5.33	4.59	−7.89	−1.47	

As mentioned above, A_{p_4} is linked with the glutinous gene as shown in table 14. On the independent Mendelian basis, the expected frequencies should be 417.87 colored non-glutinous, 139.29 colored glutinous, and 46.43 colorless glutinous. But this is far from the observed data which clearly indicate coupling between a_{p_4} and g_1. On the latter basis, the

TABLE 14

Showing coupling between a_{p_4} and g_1 from F_2 of 4269×4957.

	COLORED APEX NON-GLUTINOUS	COLORLESS APEX NON-GLUTINOUS	COLORED APEX GLUTINOUS	COLORLESS APEX GLUTINOUS	TOTAL
Observed	491	90	76	86	743
Calculated	*474.17*	*83.16*	*83.16*	*102.62*	*743*
Deviation	16.83	6.84	−7.16	−16.62	

$X^2=4.46.\quad P=0.22.$

gametic ratio is found from EMERSON'S (1916) formula, $r=10.13$, and $s=3.5$. The crossover is, therefore, about 22.34 percent.

The large deviation is clearly due to the deficiency of the glutinous plants, the latter being 2.98 times the probable error.

Further data show that A_{p4} is independent of the factors P_{r_1} and P_{r_2}, G_1 and G_2, and S_p, as is seen in tables 33, 34, and 35 respectively.

Relation between stigma color and other characters

In some varieties, the stigma is colorless, and in others it is colored with an intensity varying from pale red to dark purple. In inheritance, colored and colorless stigma segregate in different ratios according to the material used. HECTOR (1916, 1922) reported cases of 3:1, 9:7, 27:37, and 81:175 ratios, showing that there are at least five factors responsible for the production of stigma color.

The writer obtained two cases, one segregating in a 3:1 ratio, and the other, 9:7, as shown in table 15.

TABLE 15

Showing segregation of stigma color.

CASE	CROSSES COLORLESS × PURPLE	F_1	F_2 SEGREGATION		CLOSEST RATIO	F_2 EXPECTED		DEVIATION	DEV. / P. E.	SYMBOLS
			Colored	Colorless		Colored	Colorless			
1	307×410	Colored	101	41	3:1	106.5	35.5	5.5	1.58	
2	4269×4957 (ABFGI)	Colored	242	179	9:7	236.79	184.17	5.17	0.75	S_{a_1} S_{a_2}

In the first case, the factor is probably the same as A_{p_1} (or A_{p_3}), because in the same F_2 population arising from the same cross, the apiculus color alone segregated in a 9:7 ratio (see table 12) whereas a 9:3:4 ratio was observed when both apiculus and stigma were considered at the same time, as shown in table 16.

TABLE 16

Showing F_2 segregation for apex and stigma color (307×410).

	COLORED STIGMA COLORED APEX	COLORED STIGMA COLORLESS APEX	COLORLESS STIGMA COLORED APEX	COLORLESS STIGMA COLORLESS APEX	TOTAL
Observed	77	24	0	32	142
Calculated 9:3:4	*79.83*	*26.61*	*0*	*35.48*	*142*
Deviation	−2.83	−2.61	0	3.48	

In the second case, there are clearly two separate factors for the production of the stigma color. These are designated as S_{a_1} and S_{a_2}. They are complementary to each other. One of them is linked with the glutinous gene, g_1. For the sake of convenience, the linkage may be arbitrarily assumed to be between s_{a_1} and g_1. The data are presented in table 17.

TABLE 17

Showing linkage between s_{a_1} and g_1 in F_2 generation from the cross 4269×4957.

	NON-GLUTINOUS PURPLE STIGMA	NON-GLUTINOUS COLORLESS STIGMA	GLUTINOUS PURPLE STIGMA	GLUTINOUS COLORLESS STIGMA	TOTAL
Observed	210	100	32	79	421
Independent 27:21:9:7	177.39	137.97	59.13	45.99	421
Linkage	210.23	105.45	26.53	78.69	421
Deviation	−0.23	−5.45	5.47	0.31	

$$X^2=1.4094. \quad P=0.707564.$$

BRUNSON'S (1924) modified formulae

$$r=\sqrt{\frac{(AB+3ab)-(Ab+aB)}{18}}$$

$$s=\frac{1}{4}\sqrt{AB+Ab+aB+ab}-r$$

were used in calculating the intensity of the linkage between s_{a_1} and g_1. The gametic ratio is $r=4.18$ and $s=0.95$. Since this is a coupling phase, the percentage of crossing over is obtained from the formula $\frac{s}{r+s}$, namely 18.51 percent.

Data from the same cross show factors s_{a_1} and s_{a_2} are independent of p_{r_1}, p_{r_2}, g_1, g_2 and sp as shown in tables 36, 37, and 38.

The exact relation between the stigma color and the apiculus color in this particular cross is not determined. As described above, a_{p_4} is coupled with g_1, giving about 22.34 percent crossing over; and s_{a_1} is also linked with g_1, giving 18.51 percent crossing over. When the apiculus color and the stigma color were involved at the same time, a new situation arose, as shown in table 18.

The zygotic ratios calculated on the basis of three independent factors do not fit the observed ratio at all. There are, then, only two alternative explanations for the situation, namely, complete linkage between a_{p_4} and s_{a_1}, or a_{p_4} being the same factor as s_{a_1}. Since both alternatives give identical

zygotic ratio (9:3:0:4), it is impossible to decide which alternative is correct. It may be mentioned, however, that one plant (FSt 68) which is not counted in table 18 had purple stigma with a doubtful apiculus color, because the latter was under a question mark (?) in the original notebook.

TABLE 18

Relation between a_{p_4} and s_{a_1}.

	COLORED APEX PURPLE STIGMA	COLORED APEX COLORLESS STIGMA	COLORLESS APEX PURPLE STIGMA	COLORLESS APEX COLORLESS STIGMA	TOTAL
Observed	243	64	1 (FSt 68?)	117	424
Calculated 27:21:9:7	*178.74*	*139.02*	*59.58*	*46.34*	*424*
Complete linkage 9:3:0:4	238.5	79.5	0	106.0	424
Deviation	4.5	−15.5	0	11.0	

Although a_{p_4} and s_{a_1} have different crossover values with the glutinous gene, the difference is only 3.83 percent. Since both alternatives are possible, the question must be left open for the present.

Relation between the leaf-sheath color and other characters

The leaf-sheath color when present may be either self red or purple, or merely consisting of colored stripes varying in intensity. There are several genetic factors responsible for its production. PARNELL (1917) found a case where colored and colorless leaf-sheath segregated in a simple 3:1 ratio. HECTOR (1916, 1922) reported four cases segregating in 3:1, 9:7 27:37, and 15:1, respectively.

The writer has obtained two kinds of ratios, namely, 9:7 and 15:1, as shown in table 19.

TABLE 19

Showing F_2 segregation for leaf-sheath color.

CASE	CROSSES	F_1	F_2 SEGREGATION		CLOSEST RATIO	*Expected*		DEVIATION	SYMBOLS
			Colored	Colorless		Colored	Colorless		
1	4269×4957 (colorless×colored)	Colored	204	139	9:7	*192.87*	*147.01*	−8.01	Ls_1 Ls_2
2	800b_5×625 (colored×colorless)	Colored	266	14	15:1	*262.5*	*17.5*	−2.05	Ls_3 Ls_4

These data indicate that there are at least four factors which are concerned with the production of leaf-sheath color. In the first case, there must be two complementary factors, whose presence is necessary for the expression of color. These factors are designated as L_{s_1} and L_{s_2}. In the

second case, there must be two duplicate factors each of which alone can produce color. These are designated as L_{s_3} and L_{s_4}.

In the first cross, one of the complementary factors is linked with the glutinous gene. The linkage may be arbitrarily assumed to be between l_{s_1} and g_1. The gametic ratio is calculated by BRUNSON's formulae,

$$r=\sqrt{\frac{AB+3ab-(Ab+aB)}{18}}=3.73$$

$$s=\frac{1}{4}\sqrt{AB+Ab+aB+ab}-r=0.90$$

The crossing over $=\frac{s}{r+s}\times 100=19.43$ percent. By substituting the values of r and s in the following equations, we get the zygotic ratios,

$AB=9r^2+12rs+6s^2=$ colored sheath non-glutinous
$Ab=\quad 6rs+3s^2=$ colored sheath glutinous
$aB=3r^2+12rs+6s^2=$ colorless sheath non-glutinous
$ab=4r^2+\ 2rs+\ s^2=$ colorless sheath glutinous

The calculated zygotic ratios fit the observed ratios very well considering the deficiency of glutinous plants, as shown in table 20.

TABLE 20

Showing coupling between ls_1 and g_1.

	COLORED SHEATH NON-GLUTINOUS	COLORED SHEATH GLUTINOUS	COLORLESS SHEATH NON-GLUTINOUS	COLORLESS SHEATH GLUTINOUS	TOTAL
Observed	179	25	80	59	343
Calculated 27:9:21:7	*144.72*	*48.24*	*112.56*	*37.52*	*343*
Linkage (19.43% crossing over)	170.36	22.57	88.88	63.18	343
Deviation	8.64	2.43	−8.88	−4.18	

Data obtained from the same cross indicate that l_{s_1}, l_{s_2} are independent of the factors p_{r_1}, p_{r_2}, g_1, g_2, and s_p, as shown in tables 39, 40 and 41.

l_{s_1} is closely linked with a_{p_4} as shown in table 21. Since a_{p_4} is coupled with g_1, giving 22.34 percent crossing over, and since l_{s_1} is also linked with g_1, giving 19.43 percent crossing over, the order of the three genes on the glutinous chromosome would appear to be,

22.34 percent

a_{p_4} l_{s_1} 19.43 percent g_1

Linkage between stigma color and leaf-sheath color

As described above, in the F_2 generation of the cross, 4269×4957, the stigma color alone segregated in a ratio of 9 colored:7 colorless, and the leaf-sheath color also segregated in a 9:7 ratio. One of the two complemen-

TABLE 21

Showing linkage between a_{p_4} and l_{s_1} in F_2 (4269×4957).

	COLORED APICULUS PURPLE SHEATH	COLORED APICULUS COLORLESS SHEATH	COLORLESS APEX PURPLE SHEATH	COLORLESS APEX COLORLESS SHEATH	TOTAL
Observed	205	43	(BSt$_3$) 1	98	347
Expected 27:21:9:7	*146.34*	*113.82*	*48.78*	*37.94*	*346.88*
Deviation	58.66	−50.82	−47.78	60.06	

tary factors for stigma color is linked with the glutinous gene, giving 18.71 percent crossing over. One of the two complementary genes for the production of the leaf-sheath color is also linked with g_1, giving 19:43 percent crossing over. When the stigma color and the leaf-sheath color are considered at the same time, a very close linkage between the two characters is revealed, as shown in table 22a, where the calculated zygotic ratios on the basis of four independent factors are shown to be very far from the observed frequencies.

TABLE 22a

Showing close linkage between the stigma color and the leaf-sheath color.

PROGENIES	PURPLE STIGMA PURPLE SHEATH	PURPLE STIGMA COLORLESS SHEATH	COLORLESS STIGMA PURPLE SHEATH	COLORLESS STIGMA COLORLESS SHEATH	TOTAL
4269×4957B	34	2	4	23	
4269×4957D	46	3	2	29	
4269×4957F	52	4	3	44	
4269×4957I	57	0	6	37	
Observed	189	9	15	133	346
Calculated on 4 independent factors	*109.35*	*85.05*	*85.05*	*66.15*	
3 independent factors	*145.97*	*48.66*	*48.66*	*102.72*	

Since the stigma and the leaf-sheath give about the same percentage of crossing over with the glutinous gene, the difference being 0.72 percent, it is possible that the two characters have one factor in common. Most probably s_{a_1} is the same factor as l_{s_1}. For the sake of simplicity, this common factor is designated as A. Until further data demonstrate that the s_{a_1} and l_{s_1} are actually two separate entities, factor A will be considered

as a common gene which has a manifold effect conditioning stigma color as well as leaf-sheath color. On this hypothesis, then, A is complementary with S_{a_2} for stigma color and A is also complementary with L_{s_2} for leaf-sheath color.

Since the calculated ratios on the basis that A, S_{a_2} and L_{s_2} are independent factors do not fit the observed frequencies, S_{a_2} and L_{s_2} must be coupled. The situtation may be represented by the following diagram:

$$\begin{array}{|c|c|} A & a \\ Gl & g_1 \end{array} \qquad \begin{array}{|c|c|} s_{a_2} & S_{a_2} \\ l_{s_2} & L_{s_2} \end{array}$$

If $r:s:s:r$ represents any gametic series between the two linked factors, s_{a_2} and l_{s_2}, the non-crossover classes may be represented by the following equations:

$$\begin{aligned} A\ S_{a_2}\ L_{s_2} &= 3[3r^2+2(s^2+2rs)] = 9r^2+12rs+6s^2 \\ a\ S_{a_2}\ L_{s_2} &= \ 3r^2+2(s^2+2rs) \ = 3r^2+\ 4rs+2s^2 \\ A\ s_{a_2}\ l_{s_2} &= (3r^2) \qquad\qquad\quad = 3r^2 \\ a\ s_{a_2}\ l_{s_2} &= (\ r^2) \qquad\qquad\quad = \ r^2 \end{aligned}$$

On the other hand, the cross over classes may be represented as follows

$$\begin{aligned} A\ s_{a_2}\ L_{s_2} &= 3(s^2+2rs) = 6rs+3s^2 \\ A\ S_{a_2}\ l_{s_2} &= 3(s^2+2rs) = 6rs+3s^2 \\ a\ S_{a_2}\ l_{s_2} &= \ (s^2+2rs) = 2rs+s^2 \\ a\ s_{a_2}\ L_{s_2} &= \ (s^2+2rs) = 2rs+s^2 \end{aligned}$$

Since either stigma color or leaf-sheath color needs two complementary factors ($A\ S_{a_2}$ or $A\ L_{s_2}$) for its expression, the five classes ($a\ S_{a_2}\ L_{s_2}$, $A\ s_{a_2}\ l_{s_2}$, $a\ s_{a_2}\ l_{s_2}$, $a\ S_{a_2}\ l_{s_2}$, $a\ s_{a_2}\ L_{s_2}$) are thrown into the same phenotypic group By this regrouping, the four phenotypic classes are represented by the four equations:

$$\begin{aligned} XY &= 9r^2+12rs+6s^2 \quad \text{Purple stigma and purple sheath} \\ Xy &= \qquad 6rs+3s^2 \quad \text{Purple stigma and colorless sheath} \\ xY &= \qquad 6rs+3s^2 \quad \text{Colorless stigma and purple sheath} \\ xy &= 7r^2+\ 8rs+rs^2 \quad \text{Colorless stigma and colorless sheath} \end{aligned}$$

Where X = purple stigma, x = colorless stigma, Y = purple sheath and y = colorless sheath. From these equations, we get:

$$XY+xy = 16r^2+20rs+10s^2 \qquad \text{(A)}$$

$$Xy+xY = \qquad 12rs+\ 6s^2 \qquad \text{(B)}$$

Dividing both (A) and (B) by 2, we get

$$\frac{XY+xy}{2}=8r^2-10rs+5s^2 \qquad \text{(C)}$$

$$\frac{Xy+xY}{2}=\qquad 6rs+3s^2 \qquad \text{(D)}$$

Multiplying (C) by 3, and (D) by 5, we get

$$24r^2=\frac{3}{2}(XY+xy)-\frac{5}{2}(Xy+xY)$$

$$r=\sqrt{\frac{\frac{3}{2}(XY+xy)-\frac{5}{2}(Xy+xY)}{24}} \qquad (1)$$

Also,

$$XY+Xy+xY+xy=16(r^2+2rs+s^2)$$

Taking the square roots, we get

$$4(r+s)=\sqrt{XY+Xy+xY+xy}$$

$$s=\tfrac{1}{4}\sqrt{XY+Xy+xY+xy}-r \qquad (2)$$

From (1) and (2), we can calculate the gametic ratio, this being:

$$r=4.19$$
$$s=0.46$$

Since this is a coupling case, the percentage of crossing over =

$$\frac{0.46}{4.19+0.46}\times 100=9.8 \text{ percent}$$

Substituting the calculated values of r and s in the four original equations representing the four F_2 phenotypic classes, we get the frequencies quite similar to the observed ones, as shown in table 22b:

TABLE 22b

Showing close linkage between sa_2 and ls_2.

	PURPLE STIGMA PURPLE LEAF-SHEATH	PURPLE STIGMA COLORLESS SHEATH	COLORLESS STIGMA PURPLE SHEATH	COLORLESS STIGMA COLORLESS SHEATH
Observed	189	9	15	133
Calculated	*182.68*	*12.19*	*12.19*	*138.88*
Deviation	6.32	−3.19	2.81	−5.88

$$X^2=1.947$$
$$P=.584536$$

Relation between ligule color and other characters

The ligule is a structure that projects out at the juncture of the leaf-blade and the leaf-sheath. In some varieties, this structure has a deep purple color. In inheritance, the ligule color depends on several factors for its expression. HECTOR (1922) reported two cases, one segregating in 9:7 ratio and the other segregating in 27:37 ratio.

The writer has obtained a ratio of 27 purple: 37 green in the cross, 4269×4957, as shown in table 23.

TABLE 23

Showing segregation for ligule color.

	LIGULE PURPLE	LIGULE GREEN	TOTAL
Observed	109	148	257
Calculated 27:37	*108.41*	*148.55*	*257*
Deviation	0.59	−0.55	

The data clearly show that three factors are concerned in the production of ligule color in this case. These factors are designated as l_{g1}, l_{g2}, and l_{g3}. These three genes are probably independent of the factors g_1, g_1, g_2, and s_p, as shown in tables 24, 42, and 43.

TABLE 24

Showing independent segregation of g_1, l_{g1}, l_{g2}, and l_{g3}.

PROGENIES	NON-GLUTINOUS PURPLE LIGULE	NON-GLUTINOUS GREEN LIGULE	GLUTINOUS PURPLE LIGULE	GLUTINOUS GREEN LIGULE	TOTAL
4269×4957A	21	15	2	9	
4269×4957B	18	26	4	15	
4269×4957C	33	27	4	5	
4269×4957D	23	42	4	9	
Observed	95	110	14	38	257
Calculated 81:111:27:37	*81.32*	*111.44*	*27.11*	*37.15*	
Deviation	13.68	−1.44	−13.11	0.85	

Relation between ligule color and pericarp color

As mentioned above, the pericarp color in this cross is due to two duplicate factors which have been designated as P_{r_1} and P_{r_2}. When the ligule color and the pericarp color are considered at the same time, a new situation appears, as shown in table 25.

As shown in table 25, there is only one individual in the class "purple ligule and colorless pericarp" from four progenies. It is possible that some of the ligule factors are closely linked with the pericarp genes, and the rare occurrence of this class is due to linkage and the small population.

TABLE 25

Showing relation between p_{r1}, p_{r2}, l_{g1}, l_{g2}, and l_{g3}.

PROGENIES	PURPLE LIGULE COLORED PERICARP	PURPLE LIGULE COLORLESS PERICARP	GREEN LIGULE COLORED PERICARP	GREEN LIGULE COLORLESS PERICARP	TOTAL
4269×4957A	21	(Agl_3) 1	20	4	
4269×4957B	22	0	36	5	
4269×4957C	38	0	27	3	
4269×4957D	26	0	48	4	
Observed	107	1	131	16	255
Calculated 108:0:132:16	*108*	*0*	*132*	*16*	
Deviation	−1	1	−1	0	

On the other hand, it is equaly possible that one of the ligule genes is the same as one of the pericarp factors, and the single individual may be due to contamination. Indeed, the calculated ratios (108:0:132:16) on the latter basis fit the observed ratios remarkably well. However, the question must be left open for the present. Earlier investigations (HECTOR 1922) have shown a similar situation.

Relation of hull color to other characters

The term "hull" here used includes the lemma and palea which enclose the grain within. There are different colors either extended entirely over the hull such as "dark gold, ripening gold, and ripening straw," or restricted to certain portions of the hull, such as "dark furrows, piebald gold, tipped gold, patchy gold, mottled gold, and granular furrows" (PARNELL 1922). The character with which we are immediately concerned here is the ripening black color. The hull is first green as usual, but when the grains reach maturity, the hull turns black. PARNELL (1917) reported two cases of a similar condition, one segregating in a 3:1 ratio and the other 9:7.

The writer crossed two non-black varieties ($800b_5 \times 625$), the F_1 spikelets at first had green hull which later changed black or sooty black (Ridgway). The F_2 plants segregated in a ratio of approximately 9 black :7 non-black, as shown in table 26, where the data for this and other characters involved in the same cross ($800b_5 \times 625$) are presented together.

TABLE 26

Showing segregation for colors of hull, internode, leaf-sheath, apiculus, and pericarp.

	DESCRIPTION	F_2 SEGREGATION		CLOSEST RATIO	F_2 EXPECTATION		DEV. / P. E.	SYMBOLS
Hull	Black *versus* non-black	115	103	9:7	122.76	95.48	1.52	h_1 h_2
Internode	Colored *versus* colorless	142	117	9:7	145.13	112.88	0.76	In_1 In_2
Leaf-sheath	Purple *versus* colorless	266	14	15:1	262.5	17.5	0.91	ls_3 ls_4
Apiculus	Colored *versus* colorless	255	22	15:1	259.65	17.31	1.72	ap_5 ap_6
Pericarp	Colored *versus* colorless	199	19	15:1	203.42	13.56	2.25	pr_1 pr_2

The data clearly show that there are two complementary factors for the production of the black hull. These are designated as H_1 and H_2. Both these genes are independent of the factors I_{n_1}, I_{n_2}, L_{s_3}, L_{s_4}, A_{p5}, A_{p6}, P_{r_1}, and P_{r_2}, as shown in tables 27, 28, 44, and 45.

TABLE 27

Showing independent segregation between h_1, h_2, i_{n_1}, and i_{n_2}.

	BLACK HULL COLORED INTERNODE	BLACK HULL COLORLESS INTERNODE	NON-BLACK COLORED INTERNODE	NON-BLACK COLORLESS INTERNODE	TOTAL
Observed	63	47	44	57	211
Calculated 81:63:63:49	*66.74*	*51.91*	*51.91*	*40.38*	*210.94*
Deviation	−3.74	−4.91	−7.91	16.62	

The large deviation of the observed frequencies may be due to the small population, because at least 256 individuals are necessary to make the ratios barely even.

TABLE 28

Showing independent segregation between h_1, h_2, l_{s_3}, and l_{s_4}.

	BLACK HULL PURPLE SHEATH	NON-BLACK HULL PURPLE SHEATH	BLACK HULL COLORLESS SHEATH	COLORLESS HULL COLORLESS SHEATH	TOTAL
Observed	111	97	3	6	217
Calculated 135:105:9:7	*114.75*	*89.25*	*7.65*	*5.95*	*217*
Deviation	−3.75	7.75	−4.65	0.05	

DISCUSSION OF RESULTS

In the foregoing pages, data have been presented to show the behavior and chromosomal relations of twenty-five or more genetic factors affecting one or another part of the plant. To facilitate further discussion, we may outline the established genes and their behavior as follows:

FACTORS	CHARACTERS FOR WHICH THE FACTORS ARE RESPONSIBLE	RATIOS
G_1 g_1	The non-glutinous and glutinous pair	3:1
A_{n1} a_{n1} A_{n2} a_{n2}	Duplicate factors for the production of awns Independent of G_1 g_1 pair	15:1
T_y t_y	For the tawny color on the awn, apex and glumes Linked with G_1 g_1—crossover = 16.59 percent	3:1
P_{r1} p_{r1} P_{r2} p_{r2}	Duplicate factors for pericarp color Independent of g_1	15:1
G_1 g_1 G_2 g_2	Duplicate factors for the glume length Independent of g_{l1}, p_{r1} and p_{r2}	15:1
S_p s_p	Factor pair for spikelet length Independent of g_{l1}, p_{r1}, p_{r2} and g_1 Closely linked with g_2—crossing over 1.11 percent	3:1
A_{p1} a_{p1} A_{p2} a_{p2}	Complementary factors for apiculus color Independent of g_1	9:7
A_{p3} a_{p3}	A third factor for apiculus color	(27:37)
A_{p4} a_{p4}	Factor for apiculus color Linked with g_1—crossing over = 22.34 percent Independent of p_{r1}, p_{r2}, g_1, and s_p	3:1
A_{p5} a_{p5} A_{p6} a_{p6}	Duplicate factors for the apiculus color	15:1
S_{a1} s_{a1} S_{a2} s_{a2}	Complementary factors for the stigma color S_{a1} is linked with g_1—crossover = 18.51 percent Independent of p_{r1}, p_{r2}, g_1, g_2 and s_p s_{a1} may be the same factor as a_{p4} or closely linked with a_{p4}	9:7
L_{s1} l_{s1} L_{s2} l_{s2}	Complementary factors for the leaf-sheath color l_{s1} is linked with g_1—crossover = 19.43 percent They are independent of p_{r1}, p_{r2}, g_1, g_2 and s_p l_{s2} is closely linked with s_{a2}—crossing over 9.8 percent	9:7

FACTORS	CHARACTERS FOR WHICH THE FACTORS ARE RESPONSIBLE	RATIOS
L_{n_1} l_{n_1} L_{n_2} l_{n_2}	Duplicate factors for the leaf-sheath color	15:1
L_{g_1} l_{g_1} L_{g_2} l_{g_2} L_{g_3} l_{g_3}	Complementary factors for the ligule color They are independent of g_1, g_1, g_2 and s_p l_{g_3} may be the same factor as p_{r_2} or completely linked with it	27:37
H_1 h_1 H_2 h_2	Complementary factors for the black hull Independent of l_{a_3}, l_{s_4}, a_{p_6}, p_{r_1} and p_{r_2}	9:7
I_{n_1} i_{n_1} I_{n_2} i_{n_2}	Complementary factors for the internode color They are independent of h_1 and h_2	9:7

While the results outlined above are self-explanatory, a few of them may be briefly discussed with advantage. First of all, it is interesting to note that the awn, being a sporophytic character, should be either present or absent on all the spikelets of the same plant in the F_2 generation. Contrary to this expectation, two new types have appeared. One type has most of the spikelets awned, and the other has only a few spikelets awned, the remaining spikelets of the same panicle being awnless, as shown in figure 1. These new types and the original grand-parental types occur in about a 12:1:2:1 ratio. The observed frequencies have been explained on the basis of two duplicate factors. The awn may be conceived of as an extension of the central nerve of the lemma. The gene, A_{n_1}, extends the central nerve either in single or double dose with or without A_{n_2}. The gene A_{n_2} is of similar nature, but it is weaker in activity; so, the double doses may extend most of the central nerves, while the single dose may extend just a few of them. Environmental conditions may also enter in, thus influencing the action of A_{n_2} during the morphogenesis of the spikelets and particularly of the awn. As the spikelets on the top of the panicle and those on the lower part do not develop at the same time, we can easily see the differential action of the gene under different conditions in the extension of the awn. It is interesting to note that in the "mostly awned" and "rarely awned" classes, usually it is the spikelets at the lower part of the panicle whose awns are not extended.

Factorially, the case may be represented as follows:

$$\left.\begin{array}{l} 1\ A_{n_1}A_{n_1}A_{n_2}A_{n_2} \\ 2\ A_{n_1}A_{n_1}A_{n_2}a_{n_2} \\ 2\ A_{n_1}a_{n_1}A_{n_2}A_{n_2} \\ 4\ A_{n_1}a_{n_1}A_{n_2}a_{n_2} \\ 1\ A_{n_1}A_{n_1}a_{n_2}a_{n_2} \\ 2\ A_{n_1}a_{n_1}a_{n_2}a_{n_2} \end{array}\right\} = 12 \text{ fully awned}$$

1 a_{n_1} a_{n_1} A_{n_2} A_{n_2}=1 mostly awned
2 a_{n_1} a_{n_1} A_{n_2} a_{n_2}=2 rarely awned
1 a_{n_1} a_{n_1} a_{n_2} a_{n_2}=1 wholly awnless

Several factors have been shown to lie on the glutinous chromosome. T_y is coupled with G_1, giving 16.59 percent crossing over. a_{p_4} is linked with g_1, giving 22.34 percent crossing over. l_{s_1} is also coupled with g_1, giving 19.43 percent crossing over. These constitute the first linkage group which may be expressed in the following diagram:

$$a_{p_4} \quad l_{s_1} \quad t_y(?) \quad g_1 \quad t_y(?)$$

Whether t_y is on the left or right hand side of g_1 is not known at present. It may be noted that s_{a_1} is also linked with g_1, giving about 18.51 percent crossing over. However, whether s_{a_1} is the same factor as l_{s_1}, or a separate gene closely linked with l_{s_1} remains to be determined. It may be further noted that TAKAHASHI's factor (R) for awn color and YAMAGUCHI's factor (S) for apiculus color are also in the same linkage group, though their exact loci cannot be stated. It is possible that (S) is the same factor as s_{p_4}.

The second linkage group constitutes two factors, namely, s_p and g_2. The glume length depends on two duplicate factors, g_1 and g_2, segregating in a 15:1 ratio long glume being recessive. The spikelet length depends on a simple factor pair, S_p s_p, long spikelet being recessive. The factors g_2 and s_p are coupled, giving 1.11 percent crossing over. That the short spikelet factor, S_p, is coupled with one of the duplicate glume genes (G_2) is beyond doubt. It is of interest to note, however, that on the basis of 1.11 percent crossing over, only 0.99 or one plant in a population of 718 individuals is expected to have short spikelets and long glumes, and one such individual has been obtained as shown in figure 3 (F_2 SL). That this individual is a crossover is proved by the fact that there is no such variety in my stock that has short spikelets and long glumes, thus eliminating any error through contamination. It may further be pointed out that if the observed crossover plant had not appeared, the data (see table 10) could be explained equally well on the basis of 12:0:3:1 ratio by assuming that one of the duplicate glume factors is the same as the S_p factor for the spikelet length. The difficulty, however, is that on this assumption, it must follow that the long spikelet plant must necessarily have long glumes also. But this is not the case.

The third linkage group consists of s_{a_2} and l_{s_2}. The purple stigma in this case depends upon the presence of two dominant complementary factors, namely, S_{c_1} and S_{c_2}. The purple leaf-sheath also depends on two comple-

mentary factors, L_{s_1} and L_{s_2}. Since both L_{s_1} and S_{a_1} are linked with G_l, giving about the same percentage of crossing over, L_{s_1} and S_{a_1} may be the same factor. For the sake of convenience, a common factor (A) is assumed to represent both L_{s_1} and S_{a_1}, though the assumption must await further verification. It is certain, however, that linkage does exist between the characters, no matter what assumption we may make. The observed frequencies cannot be explained on the independent segregation of either four or three factors, as shown in table 22a. It appears that only on the assumption of a close linkage between S_{a_2} and L_{s_2} can we explain the observed frequencies. In so doing, the crossing over value is found to be 9.8 percent between s_{a_2} and l_{s_2}. The calculated zygotic ratios on this basis fit the observed frequencies closely.

Thus far, three linkage groups have been established beyond doubt. A fourth group is indicated by the data presented in table 25. The observed frequencies can be explained by two alternatives. One is that one of the pericarp factors is the same as one of the three ligule factors, thus giving a ratio of 108:0:132:16 on the basis of four factors, that is the two characters have one factor in common. The other alternative is that one of the pericarp factors is completely or very closely linked with one of the three ligule factors. The occurrence of one individual having "purple ligule and colorless pericarp," which cannot be accounted for by the first alternative, favors the second view. In this connection, the writer takes the liberty to rearrange HECTOR'S (1922) data in the following table for comparison with his own results:

	PURPLE LIGULE COLORED PERICARP	PURPLE LIGULE COLORLESS PERICARP	COLORLESS LIGULE COLORED PERICARP	COLORLESS LIGULE COLORLESS PERICARP	TOTAL
Observed (Hector)	1220	6	951	730	2907
Observed (Chao)	107	1	131	16	255

It must be noted, however, that though the two sets of observed frequencies are similar in nature, they are not exactly comparable. For in HECTOR'S case, the color of the pericarp depends on one factor pair segregating in a 3:1 ratio, while in the present case, the pericarp color is due to duplicate factors segregating in a 15:1 ratio. The common feature in both cases is that on the assumption of a common factor for both pericarp and ligule color, these few exceptions having "purple ligule and colorless pericarp" can not be accounted for. On the other hand, these exceptional individuals tend to support the view that one of the ligule

factors is closely linked with one of the factors responsible for the pericarp color.

Finally, it is interesting to note that so many of the characters studied are due to duplicate genes. This is of particular interest in view of the fact that all the varieties used have twenty-four chromosomes.

SUMMARY

1. Data have been presented to show the Mendelian behavior and chromosomal relations of twenty-five or more genetic factors which affect one or another part of rice plants.

2. Of the twelve characters studied, five are due to duplicate genes, each segregating in a ratio of 15:1.

3. Through the study of the interrelations between these twenty-five genes, three linkage groups have been established beyond doubt and possibly a fourth group is also indicated.

4. The first linkage group consists of four or five factors, namely, g_1, a_{p_4}, l_{s_1}, t_v, and possibly s_{a_1}.

5. There is 16.59 percent crossing over between g_1 and t_v; 18.51 percent between g_1 and s_{a_1}, 19.43 percent between g_1 and l_{s_1}, and 22.34 percent between g_1 and a_{p_4}. In addition a_{p_4} is very closely linked with l_{s_1}.

6. The second linkage group consists of two genes, namely, s_p and g_2. The glume length depends on duplicate factors, g_1 and g_2, segregating in a 15:1 ratio, long glume being recessive. The spikelet length depends on a simple factor pair, S_p s_p, long spikelet being recessive. One of the duplicate genes, presumably g_2, is coupled with sp, giving 1.11 percent crossing over.

7. The third linkage group consists of s_{a_2} and l_{s_2}. Purple stigma in this case depends upon the presence of two dominant complementary factors, namely, S_{a_1} and S_{a_2}. Purple leaf-sheath also depends on two complementary factors, L_{s_1} and L_{s_2}. Between these two characters, there is close linkage. Only on the assumption of coupling between S_{a_2} and L_{s_2} can the observed frequencies be explained. BRUNSON'S method for calculating linkage intensities involving complementary factors is further modified for the present case. The crossing over value is found to be 9.8 percent between s_{a_2} and l_{s_2}.

8. A fourth linkage group is indicated between p_{r1} or p_{r2} and one of the three complementary factors for purple ligule color.

9. Factors g_1 and g_2 are independent of g_l, p_{r_1}, and p_{r_2}.

10. The factor s_p is independent of g_l, p_{r_1}, p_{r_2}.

11. Factors a_{p_1} and a_{p_2} are independent of g_1.
12. Gene a_{p_4} is independent of p_{r_1}, p_{r_2}, g_1, g_2, and s_p.
13. Factors s_{a_1} and s_{a_2} are independent of p_{r_1}, p_{r_2}, g_1, g_2, and s_p.
14. Factors l_{s_1} and l_{s_2} are independent of p_{r_1}, p_{r_2}, g_1, g_2, and s_p.
15. Factors l_{q_1}, l_{q_2}, and l_{q_3} are independent of g_1, g_1, g_2, and s_p.
16. Factors h_1 and h_2 are independent of i_{n_1}, i_{n_2}, l_{s_3}, l_{s_4}, a_{p_5} a_{p_6}, p_{r_1}, and p_{r_2}.

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APPENDIX—TABLES 29–45

TABLE 29

Independent segregation between G_1, G_2 and G_4 from the cross (4269×4957).

PHENOTYPES	OBSERVED	*Calculated 45:3:15:1*	DEVIATION	$\frac{(O-C)^2}{C}$
Non-glutinous short glume	525	*504*	21	0.87
Non-glutinous long glume	34	*33.6*	0.4	0.05
Glutinous short glume	146	*168*	−26	2.88
Glutinous long glume	12	*11.2*	0.8	0.05
Total	717	*717*		$3.85=X^2$

$P=0.2810$

TABLE 30

Independent segregation between G_1, G_2 and P_{r_1}, P_{r_2} from the cross (4269×4957).

	SHORT GLUME COLORED PERICARP	SHORT GLUME COLORLESS PERICARP	LONG GLUME COLORED PERICARP	LONG GLUME COLORLESS PERICARP	TOTAL
Observed	590	46	42	1	679
Calculated 225:15:15:1	*596.25*	*39.75*	*39.75*	*2.85*	*679*
	−6.25	6.25	2.25	−1.65	

$P=0.495$

TABLE 31

Independent segregation between $S_p s_p$ and Gig_1 in F_2.

	SHORT SPIKELET NON-GLUTINOUS	SHORT SPIKELET GLUTINOUS	LONG SPIKELET NON-GLUTINOUS	LONG SPIKELET GLUTINOUS	TOTAL
Observed	417	118	138	40	713
Calculated 9:3:3:1	*400.5*	*133.5*	*133.5*	*44.5*	*713*
Deviation	15.5	−15.5	4.5	−4.5	

$X^2=4.34$. $P=0.2309$.

The deviation is apparently due to the large deficiency of glutinous grains.

TABLE 32

Independent segregation between factors s_p, p_{r_1}, and p_{r_2} (Ratio 45:4:15:1).

PHENOTYPES	OBSERVED	*Calculated*	$\frac{(O-C)^2}{C}$
Short spikelet, colored pericarp	505	*501.75*	0.02
Short, colorless	32	*33.45*	0.06
Long, colored	169	*167.25*	0.01
Long, colorless	8	*11.15*	0.89
	714	*714.0*	$0.98=X^2$

When $X^2=1$, $P=0.8013$.

TABLE 33

Showing independent segregation between A_{p_4} and p_{r_1} and p_{r_2}.

	COLORED APEX COLORED PERICARP	COLORLESS APEX COLORED PERICARP	COLORED APEX COLORLESS PERICARP	COLORLESS APEX COLORLESS PERICARP	TOTAL
Observed	500	162	38	9	709
Calculated 45:15:3:1	*498.15*	*166.05*	*33.21*	*11.07*	*709*
Deviation	1.85	−4.05	5.21	−2.07	

$X^2=0.982$. $P=0.801253$ when $X^2=1$.

TABLE 34

Showing independent segregation between a_{p_4} and g_1 and g_2.

	COLORED APEX SHORT GLUMES	COLORLESS APEX SHORT GLUMES	COLORED APEX LONG GLUMES	COLORLESS APEX LONG GLUMES	TOTAL
Observed	525	146	25	16	712
Calculated 45:15:3:1	*500.40*	*166.80*	*33.36*	*11.12*	*712*
Deviation	24.55	−20.80	−8.36	4.88	

$X^2=7.839$. $P=0.0418$.

The cause of the deviation is unknown.

TABLE 35

Showing independent segregation between A_{p_4} and s_p.

	COLORED APEX SHORT SPIKELET	COLORLESS APEX SHORT SPIKELET	COLORED APEX LONG SPIKELET	COLORLESS APEX LONG SPIKELET	TOTAL
Observed	428	133	139	43	743
Calculated 9:3:3:1	*417.87*	*−139.29*	*139.29*	*46.43*	
Deviation	10.13	−6.29	−0.29	−3.43	

$X^2=0.788$ $P=0.801253$ when $X^2=1$.

TABLE 36

Showing independent segregation between s_{a_1}, s_{a_2} and p_{r_1}, p_{r_2}.

	PURPLE STIGMA COLORED PERICARP	COLORLESS STIGMA, COLORED PERICARP	PURPLE STIGMA COLORLESS PERICARP	COLORLESS STIGMA, COLORLESS PERICARP	TOTAL
Observed	204	158	27	8	397
Calculated 135:105:9:7	*209.25*	*162.75*	*13.95*	*10.85*	*397*
Deviation	−5.25	−4.75	13.05	−2.85	

The deviation is probably due to the small number of plants.

TABLE 37

Showing independent segregation between s_{a_1}, s_{a_2} and g_1, g_2.

	PURPLE STIGMA COLORED PERICARP	COLORLESS STIGMA, COLORED PERICARP	PURPLE STIGMA COLORLESS PERICARP	COLORLESS STIGMA, COLORLESS PERICARP	TOTAL
Observed	227	163	12	16	418
Calculated 135:105:9:7	*220.32*	*171.36*	*14.68*	*11.42*	*418*
Deviation	6.68	−8.36	−2.68	4.58	

$X^2=2.92$. $P=0.405996$.

TABLE 38

Showing independent segregation between s_{a_1}, s_{a_2} and s_p.

	PURPLE STIGMA SHORT SPIKELET	COLORLESS STIGMA, SHORT SPIKELET	PURPLE STIGMA LONG SPIKELET	COLORLESS STIGMA, LONG SPIKELET	TOTAL
Observed	189	130	53	49	421
Calculated 27:21:9:7	*177.39*	*137.97*	*59.13*	*45.99*	*421*
Deviation	11.61	−7.97	−5.13	3.01	

$X=1.85$. $P=0.6067$.

TABLE 39

Showing independent segregation between L_{s_1}, l_{s_2} and p_{r_1}, p_{r_2} (4269×4957).

	COLORED PERICARP COLORED SHEATH	COLORED PERICARP COLORLESS SHEATH	COLORLESS PERICARP COLORED SHEATH	COLORLESS PERICARP COLORLESS SHEATH	TOTAL
Observed	179	134	19	4	336
Calculated 135:105:9:7	*177.12*	*137.76*	*11.80*	*9.18*	*336*
Deviation	1.88	−3.76	7.20	−5.18	

The deviation is probably due to the small number of plants.

TABLE 40

Showing independent segregation for l_{s_1}, l_{s_2}, g_1 and g_2.

	COLORED LEAF SHEATH SHORT GLUMES	COLORLESS SHEATH SHORT GLUMES	COLORED SHEATH LONG GLUMES	COLORLESS SHEATH LONG GLUMES	TOTAL
Observed	192	128	11	11	342
Calculated 135:105:9:7	*180.22*	*140.17*	*12.01*	*9.35*	*342*
Deviation	11.78	−12.17	−1.01	1.65	

$X^2=2.7$. $P=0.4458$.

TABLE 41

Showing independent segregation for l_{s_1}, l_{s_2} and s_p.

	COLORED LEAF SHEATH SHORT SPIKELET	COLORLESS SHEATH, SHORT SPIKELET	COLORED SHEATH LONG SPIKELET	COLORLESS SHEATH, LONG SPIKELET	TOTAL
Observed	154	100	50	39	343
Calculated 27:21:9:7	*144.72*	*112.56*	*48.24*	*37.52*	*343*
Deviation	9.28	−12.56	1.76	1.48	

$X^2=2.1$. $P=0.5543$.

TABLE 42

Showing independent segregation of g_1, g_2, l_{g_1}, l_{g_2} and l_{g_3}.

	PURPLE LIGULE SHORT GLUMES	PURPLE LIGULE LONG GLUMES	GREEN LIGULE SHORT GLUMES	GREEN LIGULE LONG GLUMES	TOTAL
Observed	104	5	134	13	256
Calculated (5 factors)	*101.25*	*6.75*	*138.75*	*9.25*	*256*
Deviation	2.75	−1.75	−4.75	3.75	

Ratio = 405:27:555:37

TABLE 43

Showing independent segregation between l_{g_1}, l_{g_2}, l_{g_3} and s_p.

	PURPLE LIGULE SHORT SPIKELET	PURPLE LIGULE LONG SPIKELET	GREEN LIGULE SHORT SPIKELET	GREEN LIGULE LONG SPIKELET	TOTAL
Observed	87	21	108	40	256
Calculated 81:27:111:37	*81*	*27*	*111*	*37*	*256*
Deviation	6	−6	−3	3	

$X^2=2.09$, $P=0.5561$.

TABLE 44

Showing independent segregation between h_1, h_2, a_{p_3} and a_{p_4}.

	BLACK HULL COLORED APICULUS	NON-BLACK COLORED APEX	BLACK HULL COLORLESS APEX	NON-BLACK COLORLESS APEX	TOTAL
Observed	109	92	5	12	218
Calculated 135:105:9:7	*114.75*	*89.25*	*7.65*	*5.95*	*218*
Deviation	−5.75	2.75	−2.65	6.05	

The deviation is probably due to the small number of plants.

TABLE 45

Showing independent segregation between h_1, h_2, p_{r_1} and p_{r_2}.

	BLACK HULL COLORED PERICARP	NON-BLACK COLORED PERICARP	BLACK HULL COLORLESS PERICARP	NON-BLACK COLORLESS PERICARP	TOTAL
Observed	106	89	7	13	215
Calculated 135:105:9:7	*113.4*	*88.2*	*7.56*	*5.88*	*215*
Deviation	−7.4	0.8	−0.56	7.12	

The deviation is probably due to the small number of plants.

LINKAGE OF THE FACTORS FOR SHORT-EAR AND DENSITY IN THE HOUSE MOUSE (*MUS MUSCULUS*)

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TABLE OF CONTENTS

	PAGE
INTRODUCTION	170
Methods	171
Results	172
DISCUSSION	177
SUMMARY	178
LITERATURE CITED	178

INTRODUCTION

In 1921 Miss LYNCH reported a mutation in the house mouse in the form of "short-ears." The ears of this mutant are short, thick, and somewhat wrinkled. Correlated with this are modifications of the skull such as shortening of the muzzle, curvature of the frontal bones, and other structural variations.

Miss LYNCH reported this as a simple recessive mutation, the gene for which was located in an autosome. The author's observations are in harmony with Miss Lynch's, for he has obtained 397 short-eared individuals out of an F_2 population of 1613, where expectation is 403, a difference of only 6 ± 11.7.

This short-eared character, from a morphological standpoint, seems similar to that described by RITZMAN (1916) in sheep. In both cases the ears are short and thick, and both are due to a single Mendelizing factor. The two characters differ, however, in their inheritance. Short-ears in sheep is dominant, while in mice it is recessive.

In February, 1925, Miss Lynch very kindly sent to Doctor W. E. CASTLE of HARVARD UNIVERSITY six short-eared males. These were mated to normal mice, mostly pink-eyed, dilute browns. The F_2 from this out-cross gave a number of short-eared individuals, from which a strain of short-eared mice was established. These were inbred for three generations and proved constant for the short-ear character.

The author wishes to thank Doctor CASTLE for the gift of these short-eared mice, which constituted the original source of his stock. He also wishes to express his appreciation of kindly suggestions of this report by Doctor CASTLE.

During the winter and spring of 1925, the author, GATES (1926), tested this short-ear mutation as to linkage relationship with the waltzing factor. It showed itself to be inherited independently of this character. KEELER (1926) found it to be independent of the factor for rodless retina. Further than this, the author is not aware that the short-eared character has been tested for linkage with any other character aside from that reported herewith.

Since the most satisfactory method of testing linkage is a back-cross to a double recessive, and since the author had a strain of pink-eyed, dilute, brown, piebald waltzers, the short-ear was crossed to this strain in the hope of establishing a strain of pink-eyed, dilute, brown, piebald, short-eared waltzers, and then crossing these with the dense black, short-eared strain, in this manner testing the short-eared character for linkage with pink-eye, dilution, brown, piebald, and waltzing at one and the same time.

It was very quickly evident, however, that there was linkage between short-ear and density, for from the first, the dilute, short-eared class was lacking, although the other recombination classes showed up as expected.

With linkage between short-ear and density apparent, the cross involving the five point recessive was abandoned so as to eliminate the unequal growth conditions in litters containing both waltzers and non-waltzers. The parent races then selected were Doctor LITTLE's strain of dilute browns, and a pink-eyed, dense, short-eared strain. I am indebted to Doctor LITTLE for kindly sending me several pens of dilute mice which were mated with the short-eared race.

The F_1 from this cross proved to be quite vigorous and hardy. The females were good mothers, seldom killing the young as was sometimes the case in the cross which involved several recessive characters including waltzing.

The character dilution involved in this experiment is that described and analyzed by Miss DURHAM (1908, 1911), LITTLE (1913), and others. It is that factor which dilutes the black color of mice to a maltese gray; and changes the chocolate brown color to a taupe. As summarized by the author, GATES (1926), this dilute factor has been shown to be inherited independently of the factors for agouti, brown, pink-eye, piebald, and waltzing. WACHTER (1927) reports it to be independent of black-eyed white.

METHODS

The mice were kept in wooden boxes with wire tops. Breeding pens were made up of not over four and usually only three females mated to

one male. Except in small litters, where there was no male, all mice of each generation were brother-sister mated.

Pregnant females were removed from the breeding pens and placed in separate boxes. The young were recorded at birth and the female left undisturbed for fourteen days. On the fourteenth day or after, the young were examined and their characters recorded.

While the characters of dilution and density are very early distinguishable (fourth to sixth day), those of short- and normal-ear cannot be determined before the young are from fourteen to sixteen days old. Occasionally, retarded growth may not enable one to distinguish the characters even at that age.

At three weeks or later, the young were weaned and their recorded characters checked. The mother was then returned to her pen. and the young mated or discarded.

All F_2 young which resembled their F_1 parents with respect to the two characters were mated *inter se* or with others like themselves. The short-eared individuals (which were all dense) were mated with the dilute and the dilute individuals with short-eared. The symbols used herewith are those adopted at New Haven, 1925, GATES (1926), namely, density—*D*, dilution—*d*, normal-ear—S^e, and short-ear—s^e.

RESULTS

When two independent Mendelian characters enter a cross they tend to segregate in the F_2 in the ratio of 9 showing both dominant characters, 3 showing one dominant and one recessive, 3 showing the other dominant and other recessive, and 1 showing both the recessive characters. Linkage will tend to disturb this ratio, and if it is quite strong, with crossing over very rare, it is possible that two cross over gametes will hardly ever unite, forming the double recessive. Complete linkage will eliminate the last class entirely and give a ratio in the other three classes of 2:1:1, in which 2 will show both dominant characters, 1 one of the recessives, and 1 the other recessive.

In this particular case the hybrid between a short-eared dense mouse and a normal-eared dilute is normal-eared and dense, as would be expected. When mated together these hybrids, if there is no linkage between short-ear and dilution, should theoretically produce four types of offspring, namely, normal-eared dense, normal-eared dilute, short-eared dense, and short-eared dilute in the ratio of 9:3:3:1.

Actually, however, these F_1 double heterozygotes produced only three types, namely, normal-eared dense, short-eared dense, and normal-eared

dilute. These have, up to this time, occurred in the ratio of 504:234:236. Theoretically, with complete linkage, the expected ratio in a population of this size would be 487:243.5:243.5, a difference of 17 ± 10.53, 9.5 ± 9.11, and 7.5 ± 9.11 respectively.

The probable error of the first class is computed on the basis of a 1:1 ratio and the other two on a 3:1 ratio of the total population. Obviously, therefore, the deviation is so slight as to be well within the range of probability of a 2:1:1 ratio. It is to be noted, however, that the deviations from a 2:1:1 ratio are all *qualitatively* such as would be expected if linkage were ***not quite*** complete, that is if a small amount of crossing over actually occurred. Thus the first class is greater than expected and the other two classes are both less than expected. However, the variations in the numbers from time to time during the experiment would indicate that the deviations were due to random sampling.

The entire absence of the fourth class indicates very close or possibly complete linkage. It indicates that if crossing over occurs at all, it is extremely rare. It suggests as a bare possibility that crossing over may in this instance be restricted to one sex, as in Drosophila. But this suggestion, aside from facts reported below, is rendered highly improbable by the repeatedly verified fact that in mice crossing over occurs almost as freely in males as in females between the genes *c* (albinism) and *p* (pinkeye,), (CASTLE 1925). The situation is similar as regards crossing over in rats, (CASTLE and WACHTER 1924), while in rabbits no significant difference is found between the sexes as regards frequency of crossing over, (CASTLE 1926).

A short-eared dilute individual would result only from a double cross over, that is, a cross over in both the gametes which united to form the zygote. The numbers are sufficiently large to make it obvious, therefore, that if crossing over is not restricted to one sex, it is so rare that there is little or no probability of two crossover gametes uniting.

From the results recorded above there is nothing to indicate that a crossover might not have taken place in one of the two gametes producing a zygote. In other words, the three phenotypes mentioned above might each possess a genetic constitution of a crossover in one of the two gametes, without altering its appearance. Thus the normal-eared dense individuals of the F_2 generation might have a genetic formula of *S*^{c}*s*^{c}*Dd*, *S*^{c}*s*^{c}*DD*, *S*^{c}*S*^{c}*Dd*, or *S*^{c}*S*^{c}*DD*. A genetic constitution indicated by either the second or the third formula would mean that a crossover had taken place in one of the two constituent gametes but not in the other, while a genetic

constitution indicated by the last formula could result only from the union of two crossover gametes.

The genetic formula of each of these normal-eared dense individuals of the F_2 generation could be determined by mating to similar sibs and noting the F_3 generation, or by mating first to a short-eared and then to a dilute individual. The former mating being simpler, and usually involving less time, and fewer offspring, was the type of mating used to test the normal-eared dense individuals of the F_2. Such matings might be of ten possible conbinations, namely:

$$S^cs^cDd \times S^cs^cDd$$
$$\text{“} \quad \times S^cS^cDd$$
$$\text{“} \quad \times S^cs^cDD$$
$$\text{“} \quad \times S^cS^cDD$$
$$S^cS^cDd \times S^cS^cDd$$
$$\text{“} \quad \times S^cs^cDD$$
$$\text{“} \quad \times S^cS^cDD$$
$$S^cs^cDD \times S^cs^cDD$$
$$\text{“} \quad \times S^cS^cDD$$
$$S^cS^cDD \times S^cS^cDD$$

A mating of the first combination, that is of double heterozygotes similar to the F_1 generation, would alone produce the three types of individuals, normal-eared dense, short-eared dense, and normal-eared dilute. Any of the nine other possible matings would produce only one, or at most two classes of young, but never three. The normal-eared dense individuals of the F_2 generation can thus, by mating *inter se*, be tested for crossovers in one or the other of the gametes which produced them.

Such matings have given the following total numbers of individuals: 177 normal-eared dense, 90 short-eared dense, and 94 normal-eared dilute. These results are so close to the 2:1:1 expectation that there is small probability of a crossover having occurred, even if these individuals had not been further tested, for on the basis of a 2:1:1 ratio, the expectation is 180.5:90.25:90.25, a difference of 3.5 ± 6.41, 0.25 ± 5.55, 3.75 ± 5.55. Here it is to be noted that qualitatively the deviations are the reverse as would occur if crossing over were an occasional or rare event, for the double dominant class is *less* than expected and the single dominant class is *greater* than expected.

Furthermore, 54 males and 66 females of normal-eared dense animals of the F_2 generation have been tested *individually* and have produced all three classes of young, showing themselves to have a genetic constitution similar to their parents of the F_1 generation. In every instance, where there have been a sufficient number of young, the parents have proved to be double heterozygotes by producing the three types of young, indicating a condition of no crossing over.

Similarly, the normal-eared dense individuals of the F_3 and F_4 generations were tested by mating *inter se*. The results of these matings show a ratio of 146:69:74, the expectations (if linkage is complete) being 144.5:72.25:72.25 respectively, and the deviations from these expectations being 1.5 ± 5.73, 3.25 ± 4.97, and 1.75 ± 4.97 respectively. Here again the results follow closely the expectation of 2:1:1 and give no indication either qualitative or quantitative of crossing over. The matings in question have been made between 35 males and 41 females of the F_3 and F_4 generations and each mating has yielded in every instance the expected three classes of young.

Since there is little or no indication from the above results that a crossover has occurred or that the normal-eared dense individuals are anything but double heterozygotes, we may combine these figures to obtain a total progeny population of 1624 for matings *inter se* of double heterozygotes, which will include 827 normal-eared dense, 393 short-eared dense and 404 normal-eared dilute, the respective expectancies being 812:406:406. Here the deviations from expectancy on the basis of 2:1:1 ratio are 15 ± 13.5, 13 ± 11.7, and 2 ± 11.7. This is a very close approximation to expectancy and tends toward the same possible qualitative result shown by the F_2 generation. But here again the results have varied from time to time and are not at all consistent. For instance, at an earlier stage in the experiment the numbers were 735:360:370 with expectation of 732.5:366.25:366.25, a deviation of 2.5 ± 12.8, 6.25 ± 11.0, and 3.75 ± 11.0 This can hardly indicate anything but random sampling.

Futhermore, combining the normal-eared dense individuals of the F_2, F_3, and F_4 generations, which have been individually tested, we find a total of 224, both males and females. In no instance where the number of offspring has been sufficient has any individual of this number failed to produce all three types of young, showing that no crossing over has occured in any of these double heterozygotes, and an indication that if crossing over occurs at all, it is a very rare event.

Each of the other two classes of the F_2 generation might just as well be the result of the union of one crossover gamete with a non-crossover.

Under these conditions the short-eared dense individuals would have a formula of $s^c s^c Dd$ and the normal-eared dilute $S^c s^c dd$, whereas the non-crossover formulae would be $s^c s^c DD$ and $S^c S^c dd$ respectively.

Both of these classes can be simultaneously tested for a crossover gamete by mating them with each other: short-eared dense with normal-eared dilute. Such matings, on the hypothesis of complete linkage, should produce only one type of young, normal-eared dense, similar in constitution to the F_1. If there should appear among the offspring a recessive individual, either short-eared or dilute, one or the other parent must have been produced by a gamete carrying a crossover. Thus, $s^c s^c Dd$ (crossover) $\times S^c S^c dd = S^c s^c Dd$ and $S^c s^c dd$ (dilute) or $S^c s^c dd$ (crossover) $\times s^c s^c DD = S^c s^c Dd$ and $s^c s^c Dd$ (short-eared). Futhermore, if this mating should produce three or four classes of young, it would indicate a crossover in one gamete of each parent, for $S^c s^c dd \times s^c s^c Dd = S^c s^c Dd + S^c s^c dd + s^c s^c Dd + s^c s^c dd$.

Up to date, 58 short-eared males and 66 short-eared females of the second, third, and fourth generations have been so tested, and in no case have the offspring been anything but normal-eared and dense. In some few instances the number of young has not been large enough to preclude the possibility of the missing class appearing among a larger population. But since expectation is half and half of each of the two possible classes in each case of a single crossover, the author has considered five offspring to constitute an adequate test. Likewise, 32 dilute males and 46 dilute females have been tested and all have proved to be homozygous for normal-ear as well as dilution. None has shown itself to have been produced by a crossover gamete.

Thus we find that both the short-eared and the dilute individuals of the F_2, F_3, and F_4 generations are homozygous for each of the two characters respectively, and therefore are like the original parental types.

Combining all these numbers we have a total of 426 individuals whose genetic constitution has been tested. Since each zygote is the product of two gametes, we may therefore conclude that 852 gametes have been tested with no case of a crossover, indicating a condition of very close or a possibility of complete linkage. Likewise, the further data afforded by the F_2, F_3, and F_4 generations tends to indicate rare or no crossing over.

Under such abnormal Mendelian ratios one must consider the possibility of lethal effects of a homozygous double recessive, therefore, at first, considerable attention was given to checking up all post-natal deaths. It was soon apparent, however, that there were no post-natal

lethal effects, for among the undepleted litters the same ratio was being obtained as among the general population. Less attention was then given to depletion of litters and the figures include both depleted and undepleted litters.

In Drosophila, MORGAN (1914) has shown that no crossing over occurs in the male. CASTLE (1925) has shown that in rats and mice crossing over is but very slightly greater in females than in males. This possibility was kept in mind in this case by testing both females and males of the F_2 generation. The results showed that all normal-eared dense individuals, both *males* and *females*, were double heterozygotes like their parents. A similar condition was found to be true in the F_3 and F_4 generations. None of those tested proved otherwise.

Likewise, both male and female short-eared individuals of the F_2, F_3, and F_4 generations were tested by mating to dilutes and no crossing over was observed in either sex. The same mating in each case gave a test for a crossover in the opposite sex.

DISCUSSION

This instance of two well established and quite different Mendelian characters showing a relationship of extremely close or complete linkage brings to mind other possible cases of complete linkage, such as the often cited instance of the blue Andalusian fowl, which LIPPINCOTT (1918, 1921) analyzed as complete linkage and not partial dominance.

Reported instances of multiple allelomorphs may prove to be, and can easily be conceived to be cases of complete linkage. Agouti pattern and yellow color have long been considered allelomorphs (Cuenot 1911). But if complete linkage can be shown to exist in other cases, it is possible that agouti and yellow are completely linked characters and not allelomorphs.

Unlike the case reported by GOWEN and GOWEN (1922), there appears to be no factor, dominant or recessive, causing this linkage, for it behaves alike on all outcrosses and there is no instance of crossing over in the F_2, F_4, or F_5 generations, nor is there any indication of crossing over in gametes which go to form heterozygotes.

DANFORTH (1927) has recently called attention to the multiple effects of the gene yellow producing yellow color and obesity. Here again the in erpretation may lie in close linkage of the gene yellow and a gene producing obesity, for the disposition to lay on fat is found in other than yellow mice, while yellow mice will nearly always do so under normal feeding conditions.

These two characters dilute and short-ear are so distinct, each of them having been bred extensively and independently for many years, that it is hardly conceivable that they are allelomorphs or that they are multiple effects of single genes, but, on the contrary, from the data here given, we are drawn to the conclusion that they are produced by two very closely linked factors. Although the number of tested gametes is quite large (852) caution should be observed in stating the degree of linkage. In rats, albinism and red-eye were at one time thought to be completely linked (IBSEN 1920), but later CASTLE and WACHTER (1924) found these to be closely linked with a very small percentage of crossing over. So here, it may be that in a larger population, cases of crossing over may occur. The present data, however, indicates that such cases would occur at least but rarely, and that the production of a short-eared dilute mouse from the present short-eared dense stock will at least involve large numbers.

SUMMARY

The data presented in this paper indicate that there is very close or possibly complete linkage between the gene short-ear and density and their allelomorphs normal-ear and dilution. No crossover has been observed in a single gamete out of a total number of 852. The F_2 generation from this cross includes only three types of individuals, normal-eared dense, short-eared dense, and normal-eared dilute, in the ratio of 2:1:1; the normal-eared dense individuals of the F_2, F_3, and F_4 generations have produced only three phenotypes. The short-eared dense individuals of the F_2, F_3, and F_4 generations when mated with normal-eared dilute sibs produce only the one phenotype—normal-eared dense, showing no crossing over, but homozygosity in every case tested.

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A COMPARISON OF THE CHROMOSOMES OF THE RAT AND MOUSE WITH REFERENCE TO THE QUESTION OF CHROMOSOME HOMOLOGY IN MAMMALS[1]

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The facts presented below owe their general interest to certain theortical considerations which have formed in part the background for a series of mammalian chromosome studies to which the writer has devoted his time for the past seven years. The question with which we are immediately concerned is this: May the chromosomes of the eutherian mammals be considered (in general) as having the same genetic make-up; for example, does the largest chromosome pair of different species carry the same genes or modifications of the same original genes? At first sight this would appear as a highly speculative problem for the cytologist to consider for, obviously, the final answer to this question lies with the geneticists of the future, but if we go on the assumption that the chromosomes represent the material substratum of genetic characters and hence in a sense that they are aggregations of genes, then it is possible for the cytologist to indicate in a provisional way the probable final answer.

The chromosome constitution of animals considered either from the standpoint of number or of size varies within wide limits; nevertheless, it has been a common experience of cytologists working in restricted fields to find that closely related species show a close similarity in their chromosome make-up. There is an easily recognized type number and deviations from this can be explained, usually, on the basis of an end-to-end fusion or to a breaking up (transverse fragmentation) of one or more chromosome pairs. The most striking example of this is found in the Acrididae which McClung and his students have studied so extensively, but animal cytology abounds with illustrations of this same condition as an examination of chromosome tables will convince any one (see HARVEY 1920, or WILSON 1925, p. 855).

The reason why chromosomes of related species are similar is undoubtedly phylogenetic, that is, related species have similar chromosome complexes because they inherited these from a common ancestor. Recent

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works in genetics and cytology have revealed unsuspected factors which would tend to stabilize this established chromosome constitution and to hold it at a constant level both quantitatively and qualitatively. The localization of sex determining factors in sex chromosomes in animals forms a very effective bar to a change in the chromosomes due to polyploidy as both the writer (PAINTER 1925a, 1925b) and MULLER (1925) have pointed out. There are various conceivable ways in which triploid or even tetraploid individuals could (and probably do) arise in nature, but such individuals could scarcely be perpetuated under natural conditions due to the complications associated with the sex chromosomes.

The loss of a chromosome pair, a whole chromosome or even a part of one chromosome due to what has been termed "genic balance" seems to be barred by the fact that such a loss is accompanied either by low fertility, sterility or low viability. Thus the absence of one of the very small fourth chromosomes in *Drosophila melanogaster* results in individuals of low fertility and viability and nullo-IV flies die. More recently GATES (1927), and the writer (1927) have shown that when only a part of one of the small chromosomes of the mouse is absent, (a normal mate being present) such individuals are extremely frail, and Gates has never been able to produce mice in which both chromosomes were affected presumably because of low viability. In this instance the chromosome involved is one of the smaller elements in a complex of forty chromosomes and represents a very small fraction of the whole chromatin mass. It thus appears that the upsetting of the normal genic balance which accompanies the loss of chromatin would probably bar the further perpetuation of such an aberrant chromosome constitution.

Just what effect the reduplication of a whole chromosome would have upon the individual is not clear (for animals) but the unstable character of the trisomic condition would require very special conditions for its perpetuation in the stable tetrasomic form, requirements which would scarcely obtain in nature.

Since the chromosomes represent directly or indirectly the physical basis of the genes, it follows that these same factors which have operated to keep the chromatin at a fixed level have also operated to stabilize the total number of gene loci and we should expect that nearly related animals having similar chromosome complexes would also show a similarity in the linkage relations of the genes. The work of METZ (1923) on Diptera, the only animal group extensively studied in this regard, seems to justify this expectation.

When the writer first began to study the chromosomes of the eutherian

mammals it was anticipated that a great variation in chromosome size and number would be encountered, but as work went forward and species from seven orders were studied it became apparent that the eutheria were much like a genus of insects in this regard. The average and the typical number for the group appears to be about 48, and deviations from this number are accompanied either by an increase in the proportion of small chromosomes, giving a higher number, or by a decrease in the proportion of small chromosomes resulting in a lower count (PAINTER 1925b). These observations accord well with the current conception that deviations from the type number in a small group can be explained on the basis of an end-to-end fusion of two or more pairs, or to a breaking in two of one or more pairs of chromosomes. If these are the only types of changes which have taken place in the eutherian orders and species then there should be an essential homology between the chromosomes of different orders and species and, what is of more interest to geneticists, a similarity in the arrangement of genes except where a break or a fusion has altered linkage values.

On the other hand, as I pointed out in a recent paper (PAINTER 1926), it is possible that, while the chromsome number has been kept at a high level in the eutheria due, perhaps, to some structural peculiarity of the eutherian cells, there may have been a great deal of shifting of chromatin within a chromsome or between non-homologous chromosomes which would greatly affect the arrangement of the genes without altering them in a quantitative way. If this shifting took place within a chromosome such as the inversion of a segment which STURTEVANT and PLUNKETT (1926) have described in *Drosophila simulans* as compared to *D. melanogaster*, it would require careful genetic tests to detect it, but if there has been a considerable shifting of chromatin between non-homologous chromosomes in related species a careful cytological study would reveal it. The extensive study of the chromosomes of rodents which has been under way at this laboratory for several years was undertaken partly with the view of gaining light upon this question.

The white rat (*Mus norvegicus*) and the common house mouse (*Mus musculus*) are placed by systematists in the same genus (*Mus*) and in view of the considerations and observations recorded above, it was anticipated that their chromosome complexes would be very similar. On the contrary, however, the complexes are very different, a fact which forces us to modify the point of view expressed in earlier mammalian chromosome studies.

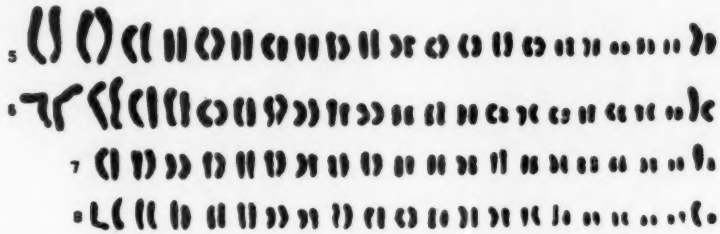
The material on which this work is based consists of the testes of rats and mice preserved by Allen's modification of Bouin's fluid and subsequently treated in just the same way. In makings drawings much time has been devoted to the careful measurement of the chromosomes, using

FIGURES.—1–4

the reflected image of the camera lucida and a pair of calipers, so that the finished drawing given below are as accurate as it has been possible to make them.

Mus norvegicus possesses 42 chromosomes and typical spermatogonial

chromosomes are shown in figures 1 and 2. These have been measured, copied and placed in serial alinement in figures 5 and 6, due consideration being given in the matching to any foreshortening observed by the microscope. The serial alinements of the elements of figures 1 and 2 (figures 5 and 6 respectively) shows that the rat possesses two pairs of very large



FIGURES.—5–8

chromosomes which are almost double the size of the third largest pair. From the third largest pair downwards the elements form a graded series. The X sex chromosome, as in other eutheria, is a medium sized chromosome but the Y is much larger than in forms heretofore studied. (The evidence for this is based on primary spermatocyte observations). The ratio of size between the smallest and largest elements (computed volume) averages about 1:8.

Mus musculus possesses 40 chromosomes and typical spermatogonial chromosomes are shown in figures 3 and 4. When these are placed in serial alinement (figures 7and 8) the chromosomes form a closely graded series, no element or pair being outstanding in size. The ratio of size between the smallest and largest (computed volume) averages about 1:5.

An attempt to express graphically the differences of the chromosomes of the two species is given in figure 9. The volume of each chromosome has been computed by measuring the length and average thickness as shown by my drawings (volume = length × thickness^2) and plotting the results in the form of a curve. Curve A (rat) and curve B (mouse)represent averages for the two alinements given for each species. In the rat (A) there is a sharp break in the curve between the second and third chromosome pairs, but from this point downward the seriation is more gradual. In the mouse (B), on the other hand, the seriation of volumes is so gradual that the curve approaches a straight line.

When one compares curves A and B and notes that except for the two large chromosome pairs of the rat, the curves are not unlike, the thought

will suggest itself that either the rat has gained most of the extra chromatin represented by the two largest pairs, or that the mouse has lost this material. It must be remembered, however, that while the unit of measure is the same in both cases (cubic mm for my drawings) that actually the rat spermatogonial cells represented are much greater in size than the

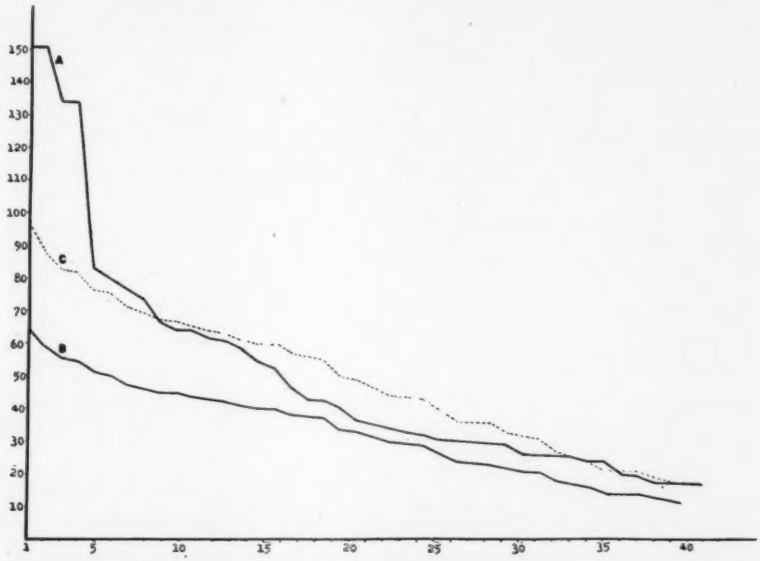


FIGURE 9.—The curves represent the volume of the chromosomes of the rat and mouse, as represented in my drawings, and measured in cubic millimeters. A is *Mus norvegicus*, B, *Mus musculus* and C, is *Mus musculus* with a correction made for difference in cell size between these two species

mouse cells, and since the size of a chromosome varies with the volume of the cell (within limits), we must correct for this difference in volume before we can legitimately draw any conclusions about gain or loss of chromatin.

To gain some idea of the relative volumes of the cells which we are comparing the large and small diameters in each case were measured, averaged and then the volumes computed assuming that the cells were spheres. The ratio of the volumes of the mouse and rat cells illustrated is 28:41. If the volume of the chromosomes, in a given tissue, is proportional to the volume of the cell, we should have to increase the volume of

the mouse chromosomes by about a half in each case to compare them directly with the rat chromosomes. This has been done in C of figure 9. Here we note that while the two large rat chromosome pairs are still far greater in volume than any of the mouse elements, that the volumes of the average sized chromosomes of the mouse are greater than in the rat. The total volume of the rat chromosomes as measured in A is 2130 cubic millimeters. The total volume of the mouse chromosomes (B) is 1327 cubic millimeters. The total volume of the mouse chromosomes as represented by C, is 1990 cubic millimeters. The difference in volume of chromatin in the rat and the mouse, when the difference in cell size is taken into account, is only about 140 cubic millimeters in favor of the rat in spite of the two pairs of large chromosomes and the higher chromosome number.

It would seem, therefore, on the basis of the measurements and calculations given above that the total volume of chromatin in the rat and in the mouse (2130 versus 1990 cubic mm for my drawings) is not very different, when differences of cell size are taken into account, and that we have no good ground for assuming that there has been any extensive loss or gain of chromatin in either species. This conclusion is to be expected, perhaps, in view of the known factors discussed in the first part of this paper which would tend to stabilize the total chromatin content in cells of related animals.

The cells selected for illustrating the chromosomes of the common rat and mouse are typical of what has been found during the past two years at this laboratory. A very large number of cells both somatic and germinal of the two species have come under observation and exact study, and the size relations illustrated have been consistently observed. The method of preservation and the subsequent treatment of the tissues has been the same in both cases. It is conceivable that rat protoplasm and chromatin might react somewhat differently from these structures in the mouse, but the reaction should be uniform, that is, to cause all the chromosomes to swell or shrink proportionally. The differences noted in the alinements of the two species can not be explained on the basis of technique.

The fact that the rat carries the higher number as well as the largest chromosomes precludes the possibility that the observed differences of the two species can be explained on the basis of an end-to-end fusion of two pairs of chromosomes (to give the mouse number) or if the mouse represents the original condition the breaking in two of one pair to give the rat number.

If there has been no great loss or gain of chromatin since the rat and mouse took their origin from a common stem, it is clear that at the present time there is a very different distribution of this material among the chromosomes of the two species. A simple shifting of material between two or three pairs of chromosomes is not adequate to make the alinements similar. If we wish to make the alinement of the rat chromosomes as represented in A of figure 9 similar to that of the mouse (C of figure 9) it would be necessary to remove a large amount of chromatin from the two largest pairs at least and to distribute this to a considerable number of medium sized rat chromosomes, a change which would involve a majority of the rat chromosomes. Or if we wish to make the mouse curve C correspond to the rat alinement, we should have to remove material from a number of medium sized mouse chromosomes and add this to the largest chromosomes. In either case the alterations necessary would be extensive and involve the majority of the chromosomes. We do not know, of course, which of the two species represents more nearly the original stem complex, indeed, the extensive differences which have been noted would suggest that both species have undergone considerable and independent changes in chromatin distribution since they originated.

The fusion of chromosome pairs and the translocation of chromatin have both been reported a number of times for animals but have been considered as comparatively rare phenomena. A comparison of the chromosomes of the rat and mouse, however, seems to indicate that these processes may have played a far more extensive role in vertebrate chromasome organization than has been heretofore thought. Such a shifting of material would not alter the genetic constitution of the animal, and hence would play no direct part in species formation, but it would throw added light upon the problem of the sterility which usually follows species crosses. That this sterility is based upon the failure of the chromosomes to mate up properly in maturation is the generally accepted and doubtless the correct explanation. With the facts just presented concerning the rat and mouse, we can understand better, perhaps, why this is the case. Just because two species look alike and are placed by systematists in the same genus, it does not follow that their chromosomes will be alike. On the contrary they may be very different as in the case immediately before us. This suggests that as a preliminary to species crosses a careful study should be made of the chromosome number and morphology in order to insure as far as this is possible that these are alike before crosses are attempted.

From the cytological standpoint the interpretation which has been given for the differences between the chromosomes of *Mus norvegicus* and *Mus musculus* lends added interest to an intensive study of the chromosomes of nearly related mammalian species in order to determine how frequent and extensive this shifting of material may have been, and how it is accomplished. Both phases are being investigated at this laboratory. At the present time it appears as possible to the writer that this change is accomplished, first, by a breaking up of the chromosomes into a larger number of smaller elements, and subsequently a fusion (without regard perhaps to previous association) of these smaller elements reducing the number again. This possibility is suggested by two sets of observations. In the first place in the wood rat common in the Austin region the chromosome number is high (about 54) and consists of relatively short chromosomes. There are no elements which correspond morphologically to the two largest pairs of chromosomes in *Mus norvegicus*. In the second place it has been observed that in the guinea pig the chromosome number is lower in prophase than in equatorial plate stages which can be interpreted as meaning either that we are witnessing in this form a breaking up of the chromosomes or a fusion of previously fragmented elements. Earlier, I was inclined to the former point of view, but the latter alternative is just as logical and seems now the more probable explanation in view of all the facts.

Since the chromatin furnishes directly or indirectly the physical substratum of the genes we should expect that the extensive redistribution of material which has taken place since the rat and mouse took their origin has entailed a rearrangement of the genes. If these two species carry approximately the same number of genes their distribution must be very different.

From a broader viewpoint the evidence which has been presented throws very grave doubt upon the idea that there is necessarily any extensive homology between the chromosomes of eutherian species in general or or even of closely related species. An extensive shifting of material between the chromsomes (such as has taken place in two closely related rodents) taken together with an inversion of a chromosome segment such as STURTEVANT and PLUNKETT have described would very quickly alter the genetic composition of any chromosome. The fact that the eutherian mammals so far studied all show a high number and a general similarity of seriation (in size) does not necessarily mean that there is any great similarity in the genetic make-up of, say, the largest chromosome. The

high number and perhaps the tendency towards a certain type of chromatin distribution may be dependent upon structural peculiarities of the eutherian cells and without any phylogenetic significance as far as the make up of the individual chromosomes is concerned.

SUMMARY

1. From considerations presented and discussed above we should expect the chromosomes of *Mus norvegicus* and *Mus musculus* to be similar. On the contrary they are very different.

2. In *Mus norvegicus* the diploid number is 42 and there are two pairs of large chromosomes nearly double the size of the next largest pair. In *Mus musculus* the diploid number is 40 and these form a closely graded series when placed in serial alinement.

3. The differences noted for the two species are too great to be accounted for on the basis of a breaking in two of a chromosome or the fusion of two pairs. It is concluded, therefore, that since the rat and mouse took their origin from a common stem there has been an extensive shifting of the chromatic material between non-homologous chromosomes, independently, in both species.

4. If the above conclusion is correct, added light is thrown on the problem of the sterility which results from species crosses and, furthermore, grave doubt must be entertained concerning the idea that there is necessarily any extensive homology between the chromosomes of different eutherian species.

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